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(54) Title: ASSAYS FOR NUCLEAR RECEPTOR LIGANDS USING FRET			
(57) Abstract <p>Provided is a method of identifying agonists and antagonists of nuclear receptors that comprises measuring agonist-dependent fluorescence resonance energy transfer (FRET) between a fluorescently labeled nuclear receptor or ligand binding domain and fluorescently labeled CREB-binding protein (CBP), p300, other nuclear co-activator, or binding portion thereof. The method is simple, rapid, and inexpensive. Nuclear receptors and nuclear receptor co-activators labeled with fluorescent reagents for use in the above-described method are also provided.</p>			

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TITLE OF THE INVENTION**ASSAYS FOR NUCLEAR RECEPTOR LIGANDS USING FRET**

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/061,385, filed 10/7/97, the contents of which are incorporated herein by reference in their entirety.

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

15

Not applicable.

FIELD OF THE INVENTION

This invention relates to methods of identifying novel agonists and antagonists of nuclear receptors utilizing the agonist-dependent interaction of such receptors with CREB-binding protein (CBP) or other nuclear receptor co-activators in which this interaction is detected by fluorescence resonance energy transfer.

BACKGROUND OF THE INVENTION

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Nuclear receptors are a superfamily of ligand-activated transcription factors that bind as homodimers or heterodimers to their cognate DNA elements in gene promoters. The superfamily, with more than 150 members, can be divided into subfamilies (e.g. the steroid, retinoid, thyroid hormone, and peroxisome proliferator-activated

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[PPAR] subfamilies). Each subfamily may consist of several members which are encoded by individual genes (e.g. PPAR α , PPAR γ , and PPAR δ). In addition, alternative mRNA splicing can result in more than one isoform of these genes as in the case of specific PPARs (e.g. PPAR γ 1 and PPAR γ 2). The nuclear receptor superfamily is involved in

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a wide variety of physiological functions in mammalian cells: e.g., differentiation, proliferation, and metabolic homeostasis. Dysfunction

or altered expression of specific nuclear receptors has been found to be involved in disease pathogenesis.

The PPAR subfamily of nuclear receptors consists of three members: PPAR α , PPAR γ , and PPAR δ . PPAR α is highly expressed in liver and kidney. Activation of PPAR α by peroxisome proliferators (including hypolipidimic reagents such as fibrates) or medium and long-chain fatty acids is responsible for the induction of acyl-CoA oxidase and hydratase-dehydrogenase (enzymes required for peroxisomal β -oxidation), as well as cytochrome P450 4A6 (an enzyme required for fatty acid ω -hydroxylase). Thus, PPAR α has an important role in the regulation of lipid metabolism and is part of the mechanism through which hypolipidimic compounds such as fibrates exert their effects. PPAR γ is predominantly expressed in adipose tissue. Recently, a prostaglandin J2 metabolite, 15-Deoxy-D12,14-prostaglandin J2, has been identified as a potential physiological ligand of PPAR γ . Both 15-Deoxy-D12,14-prostaglandin J2 treatment of preadipocytes or retroviral expression of PPAR γ in fibroblasts induced adipocyte differentiation, demonstrating the role of PPAR γ in adipocyte differentiation and lipid storage. The demonstration that anti-diabetic and lipid-lowering insulin sensitizing compounds known as thiazolidinediones are high affinity ligands for PPAR γ suggests a broad therapeutic role for PPAR γ ligands in the treatment of diabetes and disorders associated with insulin resistance (e.g. obesity and cardiovascular disease).

Nuclear receptor proteins contain a central DNA binding domain (DBD) and a COOH-terminal ligand binding domain (LBD). The DBD is composed of two highly conserved zinc fingers that target the receptor to specific promoter/enhancer DNA sequences known as hormone response elements (HREs). The LBD is about 200-300 amino acids in length and is less well conserved than the DBD. There are at least three functions for the LBD: dimerization, ligand binding, and transactivation. The transactivation function can be viewed as a molecular switch between a transcriptionally inactive and a transcriptionally active state of the receptor. Binding of a ligand which is an agonist flips the switch from the inactive state to the active state. The COOH-terminal portion of the LBD contains an activation function domain (AF2) that is required for the switch.

The ligand-induced nuclear receptor molecular switch is mediated through interactions with members of a family of nuclear receptor co-activators (e.g., CBP/p300, SRC-1/NcoA-1, TIF2/GRIP-1/NcoA-2, and p/CIP). Upon binding of agonist to its cognate receptor LBD, a conformational change in the receptor protein creates a co-activator binding surface and results in recruitment of co-activator(s) to the receptor and subsequent transcriptional activation. The binding of antagonist ligands to nuclear receptors will not induce the required conformational change and prevents recruitment of co-activator and subsequent induction of transcription. The co-activators CREB-binding protein (CBP) and p300 are two closely related proteins that were originally discovered by virtue of their ability to interact with the transcription factor CREB. These two proteins share extensive amino acid sequence homology. CBP can form a bridge between nuclear receptors and the basic transcriptional machinery (Kamei et al., 1996, Cell 85:403-414; Chakravarti et al., 1996, Nature 383:99-103; Hanstein et al., 1996, Proc. Natl. Acad. Sci. USA 93:11540-11545; Heery et al., 1997, Nature 387:733-736). CBP also contains intrinsic histone acetyltransferase activity which could result in local chromatin rearrangement and further activation of transcription. Ligand- and AF2-dependent interaction between certain nuclear receptors and CBP has been demonstrated in *in vitro* pull down assays and far-western assays. This interaction is both necessary and sufficient for the transcriptional activation that is mediated by these nuclear receptors. Thus, an AF2 mutant of the estrogen receptor (ER) which abolishes the transcriptional function of the receptor is incapable of interacting with CBP.

The N-termini of CBP and p300 have been shown to interact with the ligand-binding domains of some nuclear receptors (Kamei et al., 1996, Cell 85:403-414, hereinafter "Kamei"). Kamei was able to demonstrate direct interaction of CBP and p300 with nuclear receptors by several different methods:

(1) Kamei produced GST fusion proteins of the first 100 amino acids of the N-terminus of CBP. These fusion proteins were run out on a polyacrylamide gel, transferred to a membrane, and the membrane was exposed to ³²P-labeled ligand-binding domains of

nuclear receptors. In the presence of ligand, a specific binding interaction between the CBP and nuclear receptor fragments was detected in that the ³²P-labeled ligand-binding domains were observed to bind to the bands on the membrane containing the GST-CBP fusion proteins.

5 (2) Kamei also utilized the yeast two-hybrid system. The ligand-binding domain of the nuclear receptor fused to the DNA-binding domain of the LexA protein was used as bait. The amino terminal domain of CBP fused to the gal4 transactivation domain was used as prey. In the presence of ligand, a specific binding interaction (occurring *in vivo*, i.e., within the yeast) was observed between the CBP and nuclear receptor fragments.

10 (3) Kamei observed ligand-induced binding between CBP and nuclear receptors via a gel-shift assay. This assay is based on the observation that, in the presence of ligand, nuclear receptors will bind to oligonucleotides containing their target recognition sequence. Such binding results in the formation of a nuclear receptor-ligand-oligonucleotide complex having a higher molecular weight than the oligonucleotide alone. This difference in molecular weight is detected 15 via a shift in position of the ³²P-labeled oligonucleotide when it is run out on a polyacrylamide gel. Kamei found that a fragment of CBP (the N-terminal 100 amino acids) was capable of binding to the nuclear receptor-ligand-oligonucleotide complex and shifting the complex's position on the gel to an even higher molecular weight.

20 (4) Kamei was able to co-immunoprecipitate CBP using antibodies to nuclear receptors in extracts from a variety of cells in the presence of ligand.

25 (5) By the use of transcriptional activation assays, Kamei was able to demonstrate that nuclear receptors and CBP interact in a functional manner. Such transcriptional activation assays can indicate that two proteins are involved in a pathway that results in transcriptional activation but these assays do not prove that the interaction between the proteins is one of direct binding.

30 By the above-described methods, Kamei was able to demonstrate specific binding interactions between CBP and the retinoic acid receptor (RAR), glucocorticoid receptor (GR), thyroid hormone

receptor (T3R), and retinoid X receptor (RXR). Kamei also demonstrated specific binding between the N-terminus of p300 and RAR. However, Kamei did not demonstrate specific binding between CBP, p300, or any other nuclear receptor co-activators and PPARs.

- 5 What is striking about the methods used by Kamei is their extremely laborious and time consuming nature. Such methods involve, among other things, the construction of fusion proteins, the preparation of ³²P-labeled proteins, the construction of specialized expression vectors for the yeast two-hybrid assay and the transcriptional activation assays, the running of many gels, and the raising of antibodies. Most of these assays take days to carry out and preparing the reagents needed to carry them out may take weeks. Because of the complicated reagents that are involved in these assays and the time needed to prepare and run the assays, these assays tend to be costly.
- 10 15 Investigators other than Kamei who have studied the interaction between nuclear receptors and CBP have also been forced to rely on such cumbersome methods (see, e.g., Chakravarti et al., 1996, Nature 383:99-103; Hanstein et al., 1996, Proc. Natl. Acad. Sci. USA 93:11540-11545; Heery et al., 1997, Nature 387:733-736).
- 20 25 Kamei did not use the above-described methods to identify novel agonists or antagonists of nuclear receptors. The focus of Kamei was not on agonists or antagonists, but rather on the interaction between nuclear receptors and CBP. Although modifying the methods of Kamei to identify agonists or antagonists might be possible, such methods would suffer from serious disadvantages. This is because, as discussed above, all of the assays employed by Kamei to study the interaction of CBP and p300 with nuclear receptors are very laborious, slow, and costly. Given the therapeutic importance of steroid hormones such as estrogen, cortisol, progesterone, and other nuclear receptor agonists such as thyroid hormone and antidiabetic thiazolidinedione compounds, the need for improved high-throughput screening assays to identify potential pharmaceutical compounds affecting nuclear receptors is clear. Historically, therapeutically useful nuclear receptor ligand compounds were identified by screening animal models, an approach which is even more labor intensive and time consuming than the methods used by Kamei. Also, approaches such as those used by
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Kamei are ill-suited for the identification of antagonists of nuclear receptors. It is now widely appreciated that antagonists of nuclear receptors can be valuable therapeutic agents. Examples of such therapeutically useful antagonists are tamoxifene, raloxifene, and RU-
5 486.

What is needed is a high throughput, time and labor-saving, non-radioactive, inexpensive, and very reliable assay for the identification and characterization of both agonists and antagonists of nuclear receptors. Such an assay is provided by the present invention.
10

SUMMARY OF THE INVENTION

The present invention provides novel methods of identifying agonists and antagonists of nuclear receptors. The methods take advantage of the agonist-dependent binding of nuclear receptors and
15 CBP, p300, or other nuclear receptor co-activators. In the absence of agonist, binding between the nuclear receptor and CBP, p300, or other nuclear receptor co-activators does not occur. If agonist is present, however, such binding occurs and can be detected by fluorescence resonance energy transfer (FRET) between a fluorescently-labeled
20 nuclear receptor and fluorescently-labeled CBP, p300, or other nuclear receptor co-activator. Antagonists can be identified by virtue of their ability to prevent or disrupt the agonist-induced interaction of nuclear receptors and CBP, p300, or other nuclear receptor co-activators. In contrast to prior art methods of identifying agonists and antagonists of
25 nuclear receptors, the methods of the present invention, are simple, rapid, and less costly.

The present invention provides a nuclear receptor or ligand binding domain thereof labeled with a fluorescent reagent for use in the above-described methods of identifying agonists and antagonists of
30 nuclear receptors. The present invention also provides CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a fluorescent reagent.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a method of fluorescently labelling a protein or polypeptide with Europium cryptate (Eu³⁺K).

5 Figure 2 illustrates the format for experiments 1 and 2 of Table 1.

Figure 3 illustrates the format for experiment 3 of Table 1.

10 Figure 4 illustrates the format for experiment 4 of Table 1.

Figure 5 shows the results of studies using the methods of the present invention with four known PPAR γ agonists. -o-- = AD5075; --□-- = Pioglitazone; --×-- = Troglitazone; --◊-- = BRL49653.

15 Figure 6 shows a measurement of the binding constant for the interaction between hCBP and PPAR γ 1LBD.

Figure 7A shows the amino acid sequence of human CBP (SEQ.ID.NO.:1).

20 Figure 7B shows the nucleotide sequence of a cDNA encoding human CBP (SEQ.ID.NO.:2). The open reading frame is at positions 76-1290.

Figure 8A shows the amino acid sequence of human PPAR α (SEQ.ID.NO.:3).

25 Figure 8B shows the nucleotide sequence of a cDNA encoding human PPAR α (SEQ.ID.NO.:4). The open reading frame is at positions 217-1623.

Figure 9A shows the amino acid sequence of human PPAR γ 1 (SEQ.ID.NO.:5).

30 Figure 9B shows the nucleotide sequence of a cDNA encoding human PPAR γ 1 (SEQ.ID.NO.:6). The open reading frame is at positions 173-1609.

Figure 10A shows the amino acid sequence of human PPAR δ (SEQ.ID.NO.:7).

35 Figure 10B-C shows the nucleotide sequence of a cDNA encoding human PPAR δ (SEQ.ID.NO.:8). The open reading frame is at positions 338-1663.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

- an "agonist" is a substance that binds to nuclear receptors in such a way that a specific binding interaction between the nuclear receptor and CBP or other nuclear receptor co-activator can occur.
- 5 - an "antagonist" is a substance that is capable of preventing or disrupting the agonist-induced specific binding interaction between a nuclear receptor and CBP, p300, or another nuclear receptor co-activator.
- 10 - a "ligand" of a nuclear receptor is an agonist or an antagonist of the nuclear receptor.
- a "specific binding interaction," "specific binding," and the like, refers to binding between a nuclear receptor and CBP, p300, or other nuclear receptor co-activator which results in the occurrence of fluorescence resonance energy transfer between a fluorescent reagent bound to the nuclear receptor and a fluorescent reagent bound to CBP, p300, or other nuclear receptor co-activator.
- 15 With respect to CBP, p300, or other nuclear receptor co-activators, a "binding portion" is that portion of CBP, p300, or other nuclear receptor co-activators that is sufficient for specific binding interactions with nuclear receptors.
- 20 With respect to nuclear receptors, a "ligand binding domain" is that portion of a nuclear receptor that is sufficient to bind an agonist or antagonist of the nuclear receptor.
- 25 The present invention provides a high throughput, time and labor-saving, non-radioactive, inexpensive, and very reliable assay for the identification and characterization of both agonists and antagonists of nuclear receptors. In a general embodiment, the present invention provides methods of identifying agonists and antagonists for any nuclear receptor for which CBP, p300, or another nuclear receptor binding protein is a co-activator. Such agonists and antagonists are identified by virtue of their ability to induce or prevent binding between the ligand binding domain of a nuclear receptor and CBP, p300, or other nuclear receptor co-activator. The interaction between the nuclear receptor and CBP, p300, or other nuclear receptor co-activator is monitored by observing the occurrence of fluorescence resonance energy
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- 35

transfer (FRET) between two fluorescent reagents. One fluorescent reagent is bound to the nuclear receptor; the other fluorescent reagent is bound to CBP, p300, or other nuclear receptor co-activator. The binding of fluorescent reagent to nuclear receptor, CBP, p300, or other nuclear receptor co-activator can be by a covalent linkage or a non-covalent linkage.

The present invention makes use of fluorescence resonance energy transfer (FRET). FRET is a process in which energy is transferred from an excited donor fluorescent reagent to an acceptor fluorescent reagent by means of intermolecular long-range dipole-dipole coupling. FRET typically occurs over distances of about 10 \AA to 100 \AA and requires that the emission spectrum of the donor reagent and the absorbance spectrum of the acceptor reagent overlap adequately and that the quantum yield of the donor and the absorption coefficient of the acceptor be sufficiently high. In addition, the transition dipoles of the donor and acceptor fluorescent reagents must be properly oriented relative to one another. For a review of FRET and its applications to biological systems, see Clegg, 1995, Current Opinions in Biotechnology 6:103-110.

The present invention makes use of a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent. The second fluorescent reagent comprises a fluorophore capable of undergoing energy transfer by either (a) donating excited state energy to the first fluorescent reagent, or (b) accepting excited state energy from the first fluorescent reagent. In other words, according to the present invention, either the first or the second fluorescent reagents can be the donor or the acceptor during FRET.

The first and second fluorescent reagents are spectropscopically complementary to each other. This means that their spectral characteristics are such that excited state energy transfer can occur between them. FRET is highly sensitive to the distance between the first and second fluorescent reagents. For example, FRET varies inversely with the sixth power of the distance between the first and second fluorescent reagents. In the absence of agonist, the first

fluorescent reagent, bound to the nuclear receptor or ligand binding domain thereof, will not be near the second fluorescent reagent, bound to CBP, p300, or other nuclear receptor co-activator, or binding portion thereof. Thus, no FRET, or very little FRET, will be observed. In the

5 presence of agonist, however, interaction between the nuclear receptor and CBP, p300, or other nuclear receptor co-activator will occur, thus bringing close together the first and the second fluorescent reagents, allowing FRET to occur and be observed.

Accordingly, the present invention provides a method of
10 identifying an agonist of a nuclear receptor that comprises providing:

- (a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;
- (b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent; and
- 15 (c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, will occur; and

20 (d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

25 In particular embodiments, the nuclear receptor is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, and "orphan nuclear receptors" such as LXR, FXR, etc.

30 In a particular embodiment, the nuclear receptor or ligand binding domain thereof is a full-length nuclear receptor. In another embodiment, the nuclear receptor or ligand binding domain thereof is a ligand binding domain of a nuclear receptor. In another embodiment, the nuclear receptor or ligand binding domain thereof comprises an AF-2 site of a nuclear receptor.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is a full-length PPAR. In another embodiment, the nuclear receptor or ligand binding domain thereof is the ligand binding domain of a PPAR. In a further embodiment, the PPAR is selected from the group consisting of PPAR α , PPAR γ 1, PPAR γ 2, and PPAR δ . In a further embodiment, the ligand binding domain of the PPAR contains amino acid residues 176-478 of human PPAR γ 1.

- In a particular embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 143-462 of human RAR α .
- 10 In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 122-410 of rat T3R α 1. In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 227-463 of mouse RXR γ . In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 251-595 of human ER.

In a particluar embodiment, the above-described methods utilize full-length CBP, either mouse or human. In other embodiments, the methods utilize amino acid residues 1-113 of human CBP. In another embodiment, the methods utilize amino acid residues 1-453 of human CBP.

The conditions under which the methods described above are carried out are conditions that are typically used in the art for the study of protein-protein interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used buffers as PBS; a temperature of about 4°C to about 55°C. The presence of commonly used non-ionic detergents, e.g., NP-40®, sarcosyl, Triton X-100®, is optional. When europium cryptates are used as fluorescent reagents, reactions should contain KF at a concentration of at least 200 mM.

Heery et al., 1997, Nature 387:733-736 showed that interactions between nuclear receptors and a variety of nuclear receptor co-activators are mediated by a short amino acid sequence in the nuclear receptor co-activators having the amino acid sequence LXXLL, where L is leucine and X represents any amino acid. Accordingly, the present invention can be practiced with a binding portion of a nuclear receptor co-activator, provided that the binding portion contains the amino acid

sequence LXXLL. Therefore, the present invention includes a method of identifying an agonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

5 (b) a binding portion of a nuclear receptor co-activator, where the binding portion contains the amino acid sequence LXXLL, and where the binding portion is labeled with a second fluorescent reagent; and

10 (c) a substance suspected of being an agonist of the nuclear receptor;

under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the binding portion of the nuclear receptor co-activator will take place; and

15 (d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;

where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

20 In a particular embodiment, the nuclear receptor co-activator is selected from the group consisting of: human RIP-140, human SRC-1, mouse TIF-2, human or mouse CBP, human or mouse p300, mouse TIF-1, and human TRIP proteins.

25 In a particular embodiment, the nuclear receptor co-activator is human RIP-140 and the binding portion includes a contiguous stretch of amino acids of human RIP-140 selected from the group consisting of: positions 20-29, 132-139, 184-192, 266-273, 379-387, 496-506, 712-719, 818-825, 935-944, and 935-942.

30 In another embodiment, the nuclear receptor co-activator is human SRC-1 and the binding portion includes a contiguous stretch of amino acids of human SRC-1 selected from the group consisting of: positions 45-53, 632-640, 689-696, 748-755, and 1434-1441.

35 In another embodiment, the nuclear receptor co-activator is mouse TIF-2 and the binding portion includes a contiguous stretch of amino acids of mouse TIF-2 selected from the group consisting of: positions 640-650, 689-699, and 744-754.

In another embodiment, the nuclear receptor co-activator is human or mouse CBP and the binding portion includes a contiguous stretch of amino acids of human or mouse CBP selected from the group consisting of: positions 68-78 and 356-366.

5 In another embodiment, the nuclear receptor co-activator is human or mouse p300 and the binding portion includes a contiguous stretch of amino acids of human or mouse p300 selected from the group consisting of: positions 80-90 and 341-351.

10 In another embodiment, the nuclear receptor co-activator is mouse TIF-1 and the binding portion includes a contiguous stretch of amino acids of mouse TIF-1 containing positions 722-732.

In another embodiment, the nuclear receptor co-activator is human TRIP2 and the binding portion includes a contiguous stretch of amino acids of human TRIP2 containing positions 23-33.

15 In another embodiment, the nuclear receptor co-activator is human TRIP3 and the binding portion includes a contiguous stretch of amino acids of human TRIP3 containing positions 97-107.

20 In another embodiment, the nuclear receptor co-activator is human TRIP4 and the binding portion includes a contiguous stretch of amino acids of human TRIP4 containing positions 36-46.

In another embodiment, the nuclear receptor co-activator is human TRIP5 and the binding portion includes a contiguous stretch of amino acids of human TRIP5 containing positions 26-36.

25 In another embodiment, the nuclear receptor co-activator is human TRIP8 and the binding portion includes a contiguous stretch of amino acids of human TRIP8 containing positions 36-46.

In another embodiment, the nuclear receptor co-activator is human TRIP9 and the binding portion includes a contiguous stretch of amino acids of human TRIP9 selected from the group consisting of: positions 73-83, 256-266 and 288-298.

30 For amino acid sequences of nuclear receptor co-activators, see Yao et al., 1996, Proc. Natl. Acad. Sci. USA 93:10626-10631 (SRC-1); O'Sate et al., 1995, Science 270:1354-1357 (SRC-1); Cavaillès et al., 1995, EMBO J. 14:3741-3751 (RIP-140); Voegel et al., 1996, EMBO J. 15:101-108 (TIF-2); Kwok et al., 1994, Nature 370:223-226 (CBP); Arias et al., 1994, Nature 370:226-229 (CBP); Eckner et al., 1994, Genes Dev. 8:869-884

(p300); Le Douarin et al., 1995, EMBO J. 14:2020-2033 (TIF-1); Lee et al., 1995, Nature 374:91-94 (TRIP proteins).

5 The particular embodiments of the present invention described above are all particular embodiments of a more general method that is also part of the present invention. That general method is a method of identifying an agonist of a nuclear receptor that comprises providing:

- 10 (a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;
- 10 (b) a polypeptide containing the amino acid sequence LXXLL where the polypeptide is labeled with a second fluorescent reagent; and
- 15 (c) a substance suspected of being an agonist of the nuclear receptor;
15 under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the polypeptide will take place; and
- 20 (d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;
20 where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.

25 In a particular embodiment, the amino acid sequence LXXLL is present in an α helical portion of the polypeptide. In another embodiment, the amino acid sequence LXXLL is present in an α helical portion of the polypeptide and the leucines form a hydrophobic face.

30 The present invention provides methods for identifying antagonists of a nuclear receptor. Such methods are based on the ability of the antagonist to prevent the occurrence of agonist-induced binding between a nuclear receptor and CBP, p300, or other nuclear receptor co-activator, or to disrupt such binding after it has occurred. Thus, the present invention provides a method for identifying antagonists of nuclear receptors that comprises providing:

- 35 (a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;
- (b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent;

- (c) an agonist of the nuclear receptor; and
- (d) a substance suspected of being an antagonist of the nuclear receptor;

under conditions such that, in the absence of the substance,
5 binding between the nuclear receptor or ligand binding domain thereof
and CBP, p300, or other nuclear receptor co-activator, or a binding
portion thereof will occur; and

(e) measuring fluorescence resonance energy transfer
(FRET) between the first and second fluorescent reagents when the
10 substance is present and measuring FRET between the first and second
fluorescent reagents when the substance is absent;

where the a decrease in FRET when the substance is
present indicates that the substance is an antagonist of the nuclear
receptor.

15 In particular embodiments, the nuclear receptor is selected
from the group consisting of steroid receptors, thyroid hormone
receptors, retinoic acid receptors, peroxisome proliferator-activated
receptors, retinoid X receptors, glucocorticoid receptors, vitamin D
receptors, and "orphan nuclear receptors" such as LXR, FXR, etc.

20 In a particular embodiment, the nuclear receptor or ligand
binding domain thereof is a full-length nuclear receptor. In another
embodiment, the nuclear receptor or ligand binding domain thereof is a
ligand binding domain of a nuclear receptor. In another embodiment,
the nuclear receptor or ligand binding domain thereof is an AF-2 site of
25 a nuclear receptor.

In a particular embodiment, the nuclear receptor or ligand
binding domain thereof is a full-length PPAR. In another embodiment,
the nuclear receptor or ligand binding domain thereof is the ligand
binding domain of a PPAR. In a further embodiment, the PPAR is
30 selected from the group consisting of PPAR α , PPAR γ , and PPAR δ . In a
further embodiment, the ligand binding domain of the PPAR contains
amino acid residues 176-478 of human PPAR γ 1.

35 In a particular embodiment, the nuclear receptor or ligand
binding domain thereof contains amino acids 143-462 of human RAR α .
In another embodiment, the nuclear receptor or ligand binding domain
thereof contains amino acids 122-410 of rat T₃R α 1. In another

embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 227-463 of mouse RXR γ . In another embodiment, the nuclear receptor or ligand binding domain thereof contains amino acids 251-595 of human ER.

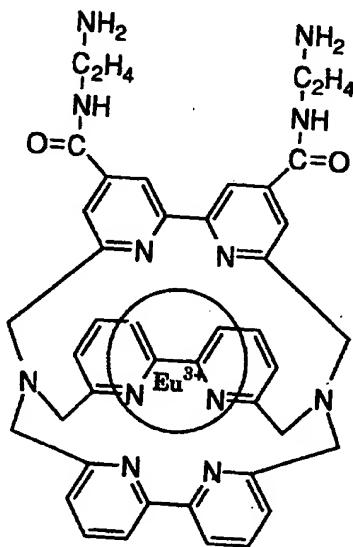
5 In a particular embodiment, the above-described methods utilize full-length CBP, either mouse or human. In other embodiments, the methods utilize amino acid residues 1-113 of human CBP. In another embodiment, the methods utilize amino acid residues 1-453 of human CBP.

10 The conditions under which the methods described above are carried out are conditions that are typically used in the art for the study of protein-protein interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used buffers as PBS; a temperature of about 4°C to about 55°C. The presence of
15 commonly used non-ionic detergents, e.g., NP-40®, sarcosyl, Triton X-100®, is optional. When europium cryptates are used as fluorescent reagents, reactions should contain KF at a concentration of at least 200 mM.

20 In principle, one could measure FRET by monitoring either (a) a decrease in the emission of the donor fluorescent reagent following stimulation at the donor's absorption wavelength and/or (b) an increase in the emission of the acceptor reagent following stimulation at the donor's absorption wavelength. In practice, FRET is most effectively measured by emission ratioing. Emission ratioing monitors the change
25 in the ratio of emission by the acceptor over emission by the donor. An increase in this ratio signifies that energy is being transferred from donor to acceptor and thus that FRET is occurring. Emission ratioing can be measured by employing a laser-scanning confocal microscope. Emission ratioing is preferably done by splitting the emitted light from a
30 sample with a dichroic mirror and measuring two wavelength bands (corresponding to the donor and the acceptor emission wavelengths) simultaneously with two detectors. Alternatively, the emitted light can be sampled consecutively at each wavelength (by using appropriate filters) with a single detector. In any case, these and other methods of measuring FRET are well known in the art.

Although a variety of donor and acceptor fluorescent reagents can be used in the practice of the present invention, preferred embodiments of the present invention make use of cryptates of fluorescent reagents as donor reagents. Inclusion of a substrate into the intramolecular cavity of a macropolycyclic ligand results in the formation of a cryptate. The macropolycyclic ligand shields the substrate from interaction with solvent and other solute molecules. If the substrate is a fluorescent reagent, formation of a cryptate may result in markedly different spectroscopic characteristics for the reagent as compared to the spectroscopic characteristics of the free reagent.

The present invention includes the use of europium (Eu^{III}) or terbium (Tb^{III}) cryptates as donor fluorescent reagents. Such Eu^{III} or Tb^{III} cryptates, as well as methods for their formation, are well known in the art. For example, see Alpha et al., 1987, Angew. Chem. Int. Ed. Engl. 26:266-267; Mathis, 1995, Clin. Chem. 41:1391-1397. A europium cryptate is formed by the inclusion of a europium ion into the intramolecular cavity of a macropolycyclic ligand which contains bipyridine groups as light absorbers. When europium cryptates are present in solution together with fluoride ions, a total shielding of the europium cryptate fluorescence occurs. The molecular structure of a europium cryptate is shown below.



Europium cryptates can be conjugated to proteins by the use of well-known heterobifunctional reagents (see, e.g., International Patent Application WO 89/05813; Prat et al., 1991, Anal. Biochem. 195:283-289; Lopez et al., 1993, Clin. Chem. 39:196-201).

- 5 The present invention includes the use of XL665 as the acceptor fluorescent reagent. XL665 is a crosslinked derivative of allophycocyanin (APC). APC is a porphyrin containing protein which is derived from the light harvesting system of algae (Kronick, 1986, M. Immunol. Meth. 92:1-13). XL665 has an absorption maximum at ~620 nm and an emission maximum at 665 nm. In some embodiments of the invention, XL665 is labeled with streptavidin in order to effect the binding of the streptavidin-labeled XL665 to a biotin-labeled substance, e.g., CBP or the ligand binding domain of a nuclear receptor. Streptavidin labeling of XL665 and biotin labeling of CBP, or the ligand binding domain of a nuclear receptor, can be performed by well known methods.
- 10 15

In a preferred embodiment of the invention, XL665 as the acceptor fluorescent reagent is combined with Europium cryptate (Eu³⁺K) as the donor fluorescent reagent. Europium cryptate (Eu³⁺K) has a large Stokes shift, absorbing light at 337 nm and emitting at 620 nm. Thus, the emission maximum of Europium cryptate (Eu³⁺K) overlaps the absorption maximum of XL665. Europium cryptate (Eu³⁺K) has a large temporal shift; the time between absorption and emission of a photon is about 1 millisecond. This is advantageous because most background fluorescence signals in biological samples are short-lived. Thus the use of a fluorescent reagent such as europium cryptate, with a long fluorescent lifetime, permits time-resolved detection resulting in the reduction of background interference.

- 20 25
- 30 35
- The spectral and temporal properties of europium cryptate (Eu³⁺K) result in essentially no fluorescence background and thus assays using this fluorescent reagent can be carried out in a "mix and read" mode, greatly facilitating its use as a high throughput screening tool. For the embodiment using Europium cryptate (Eu³⁺K) and XL665, the measuring instrument irradiates the sample at 337 nm and measures the fluorescence output at two wavelengths, 620 nm (B counts, europium fluorescence) and 665 nm (A counts, XL665 fluorescence).

The extent of fluorescent resonance energy transfer is measured as the ratio between these two values. Typically this ratio is multiplied by 10,000 to give whole numbers.

Other FRET donor-acceptor pairs are suitable for the
5 practice of the present invention. For example, the following donor-acceptor pairs can be used: dansyl/fluorescein; fluorescein/rhodamine; tryptophan/aminocoumarin.

The present invention provides a nuclear receptor or ligand binding domain thereof labeled with a fluorescent reagent for use in the
10 above-described methods of identifying agonists and antagonists of nuclear receptors. The present invention also provides CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a fluorescent reagent.

In a particular embodiment, the nuclear receptor or ligand binding domain thereof is selected from the group consisting of PPAR α , PPAR γ , PPAR δ , a ligand binding domain of PPAR α , PPAR γ , or PPAR δ , and amino acid residues 176-478 of human PPAR γ 1 and the fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

20 In a particular embodiment, CBP, p300, or other nuclear receptor co-activator is labeled with a fluorescent reagent selected from the group consisting of XL665 and Europium cryptate (Eu3+K).

The following non-limiting examples are presented to better
25 illustrate the invention.

EXAMPLE 1

Cloning, expression, and purification of human CBP and PPAR proteins

To test whether human CBP can interact with PPARs in an
30 agonist-dependent manner, we cloned the human cDNA fragments encoding the NH₂-terminal 1-113 amino acids (hCBP1-113) and 1-453 amino acids (hCBP1-453) of human CBP by the polymerase chain reaction (PCR). The DNA and amino acid sequences of human CBP are

disclosed in Borrow et al., 1996, *Nature Genet.* 14:33-41 and in GenBank, accession no. U47741.

The primers used for hCBP1-113 were:

- 5' -ACTCGGATCCAAGCCATGGCTGAGAACTTGCTGGACGG-3'
5 (SEQ.ID.NO.:9) and
5'-CACAAAGCTTAGGCCATGTTAGCACTGTTGG-3' (SEQ.ID.NO.:
10).

These primers were expected to amplify a 0.9 kb DNA fragment.

The primers for hCBP1-453 were:

- 10 5'-ACTCGGATCCAAGCCATGGCTGAGAACTTGCTGGACGG-3'
(SEQ.ID.NO.:9) and
5'CTCAGTCGACTTATTGAATTCCACTAGCTGGAGATCC-3'
(SEQ.ID.NO.:11).

These primers were expected to amplify a 1.5 kb DNA fragment..

- 15 The template for the PCR reaction was a human fetal brain cDNA library (Stratagene, Catalogue #IS 937227). Of course, any human cDNA library from a tissue expressing CBP could have been used. The PCR amplified 0.9 kb and 1.5 kp DNA fragments which were digested with restriction endonucleases and ligated into pBluescript II
20 vector. DNA sequencing analysis confirmed that the amplified fragments were identical to the corresponding published nucleic acid sequences of human CBP.

- Based on the publicly available sequences for human CBP cited above, other primers could be readily identified and prepared by those skilled in the art in order to amplify and clone other portions of cDNA encoding human CBP from appropriate cDNA libraries. Once such portions of human CBP are produced, they could be used in the methods of the present invention in a manner similar to that described herein for hCBP1-113 and hCBP1-453. The amino acid sequence of human CBP is shown in Figure 7A; the nucleic acid sequence of the cDNA encoding human CBP is shown in Figure 7B.

- 30
35 To express the polypeptides encoded by the PCR fragments, vectors encoding fusion proteins of the polypeptides and glutathione S-transferase (GST) were constructed and expressed in *E. coli*. The PCR fragments were subcloned into the expression vector pGEX (Pharmacia Biotech) to generate pGEXhCBP1-113 and pGEXhCBP1-453.

pGEXhCBP1-113 and pGEXhCBP1-453 were transfected into the DH5 α strain of *E. coli* (GIBCO BRL) and the bacteria hosting either pGEXhCBP1-113 or pGEXhCBP1-453 were cultured in LB medium (GIBCO BRL) to a density of OD₆₀₀ = 0.7-1.0 and induced for

- 5 overexpression of the GST-CBP fusion proteins by addition of IPTG (isopropylthio- β -galactoside) to a final concentration of 0.2 mM. The IPTG induced cultures were further grown at room temperature for 2-5 hrs. The cells were harvested by centrifugation for 10 min at 5000g. The cell pellet was used for GST-CBP fusion protein purification by following
- 10 the procedure from Pharmacia Biotech using Glutathione Sepharose beads. hCBP1-113 and hCBP1-453 proteins were generated by cleaving the corresponding GST fusion proteins with thrombin. SDS-polyacrylamide gel electrophoresis analysis showed that the preparation from pGEXhCBP1-113 gave two polypeptide bands, with apparent
- 15 molecular weight of 12 kd and 10 kd. The 12 kd band is the expected size of hCBP1-113 and the 10 kd band is most likely a premature translational termination product. The preparation from pGEXhCBP1-450 gave a single band with the expected size, 50 kd.

- cDNAs encoding full-length PPAR α and PPAR γ 1 were
- 20 subcloned into pGEX vectors for the production of GST-PPAR α and GST-PPAR γ 1 fusion proteins in *E.coli*. PPAR γ 1 was cloned from a human fat cell cDNA library (see Elbrecht et al., 1996, Biochem. Biophys. Res. Comm. 224:431-437). A cDNA encoding the human PPAR γ 1 ligand binding domain (PPAR γ 1LBD; amino acids 176-478 of PPAR γ 1) was
 - 25 subcloned from a modified pSG5 vector as a Xho I (site located in the N-terminus of the LBD)/ Xba I (site located in the pSG5 vector) fragment. The Xba I site was blunt-ended with T4 DNA polymerase. The 1.1 kb fragment containing the LBD was purified from an agarose gel and ligated into pGEX-KG (see Guan & Dixon, 1991, Anal. Biochem. 192:262-
 - 30 267) that had been digested with Xho I and Hind III (the Hind III site had been blunt-ended with T4 DNA polymerase). This construct was used for the production of GST-hPPAR γ 1LBD and hPPAR γ 1LBD (the ligand binding domain cleaved free of GST). The overexpression and purification of PPAR α , PPAR γ 1, and PPAR γ 1LBD were as described
 - 35 above for CBP.

The DNA and amino acid sequences of human PPAR α are disclosed in Schmidt et al., 1992, Mol. Endocrinol. 6:1634-1641 and in GenBank, accession no. L07592. See Figure 8A and 8B.

5 The DNA and amino acid sequences of human PPAR γ 1 are disclosed in Greene et al., 1995, Gene Expr. 4:281-299; Qi et al., 1995, Mol. Cell. Biol. 15:1817-1825; Elbrecht et al., 1996, Biochem. Biophys. Res. Comm. 224:431-437; and in GenBank, accession no. L40904. See Figure 9A and 9B. Human PPAR γ 2 contains the same amino acid sequence as human PPAR γ 1 except for an amino terminal addition of 24 amino acids
10 (see Elbrecht et al., 1996, Biochem. Biophys. Res. Comm. 224:431-437). Thus, the amino acid sequence of the ligand binding domain of human PPAR γ 2 is the same as the amino acid sequence of the ligand binding domain of human PPAR γ 1, although the numbering of the amino acids differs (176-478 for human PPAR γ 1 and 200-502 for human PPAR γ 2).

15 The DNA and amino acid sequences of human PPAR δ are disclosed in Sher et al., 1993, Biochemistry 32:5598-5604 and in GenBank, accession no. L02932. See Figure 10A-C.

EXAMPLE 2

20 Interaction between PPARs and hCBP fragments

Experiments were first conducted using hCBP1-113 and hPPAR γ 1LBD. Purified hPPAR γ 1LBD was biotinylated with Sulfo-NHS-LC-Biotin (PIERCE) to a biotin:hPPAR γ 1LBD ratio of 3:1 according to the procedure provided by PIERCE. Purified hCBP1-113 was directly labeled with europium cryptate (Eu3+K) by the method illustrated in Figure 1. Biotin-labeled hPPAR γ 1LBD, Eu3+K-labeled hCBP1-113, and streptavidin-labeled XL665 (SA-XL665; from PACKARD) were incubated together in the presence or absence of 1 μ M of known PPAR γ agonist (BRL49653 or AD5075).

30 Thus, this experimental format made use of the fluorescent reagent pair europium cryptate (Eu3+K), which acted as donor, and XL665, which acted as acceptor. hCBP1-113 was directly labeled with europium cryptate (Eu3+K); hPPAR γ 1LBD was indirectly labeled with XL665 by means of a biotin-streptavidin link. The emission maximum

of europium cryptate (Eu³⁺K) overlaps with the absorption maximum of XL665. Therefore, when europium cryptate (Eu³⁺K) and XL665 are in close proximity, and the sample is illuminated with light at 337 nm (the absorption maximum of europium cryptate (Eu³⁺K)), FRET can occur

5 between europium cryptate (Eu³⁺K) and XL665. This FRET manifests itself as increased emission at 665 nm by XL665. Figure 2 shows a schematic of the format used in this experiment (experiment 1 of Table 1). When agonist is bound to hPPAR γ 1LBD, a specific interaction occurs between hPPAR γ 1LBD and hCBP1-113, thus bringing europium cryptate

10 (Eu³⁺K) and XL665 into close enough proximity for FRET to occur. In the absence of agonist, no interaction occurs between hPPAR γ 1LBD and hCBP1-113 and thus europium cryptate (Eu³⁺K) and XL665 are not brought into close proximity and no FRET occurs. When FRET occurs, the amount of light given off by the sample at the emission maximum of

15 XL665 (665 nm) is increased relative to the amount of light given off by the sample at the emission maximum of europium cryptate (Eu³⁺K) (620 nm). Therefore, measuring the ratio of emission at 665 nm to 620 nm in the presence and the absence of a substance suspected of being an agonist allows for the determination of whether that substance actually

20 is an agonist. If the substance is an agonist, an increase in the ratio of emission at 665 nm to 620 nm in the presence of the substance will be observed.

Reactions were carried out in microtiter plates. Reaction conditions were: appropriate volume (total 250 μ l) of the reaction buffer (either PBS or HEPES, see below, containing 500 mM KF, 0.1% bovine serum albumin, BSA) was added to each well, followed by addition of ligands (BRL49653 or AD5075 at a final concentration of 1 μ M and 0.1% dimethylsulfoxide (DMSO) or vehicle control (0.1% DMSO), Eu³⁺K labeled hCBP (100 nM), biotin-hPPAR γ 1LBD (100 nM), and streptavidin-labeled XL665 (100 nM) to appropriate wells. After mixing, 200 μ l of reaction mixture was transferred to a new well. The plate was either directly measured for fluorescence resonance energy transfer (FRET) or covered with sealing tape (PACKARD) to avoid evaporation and incubated at room temperature for up to 24 hrs before measuring FRET.

The results of this experiment and others described below yielded ratio values as follows:

Table 1

Experiment	Buffer	Emission ratio with AD5075	Emission ratio with vehicle
1	PBS	1134	1074
2	HEPES + 0.05% NP40	967	617
3	HEPES + 0.05% NP40	1078	536
4	HEPES + 0.05% CHAPS	1883	487

Experiment 1 of Table 1 was carried out using PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, pH 7.4). The greater emission ratio observed in the presence of AD5075 demonstrated that a specific interaction between hCBP1-113 and hPPAR γ 1LBD

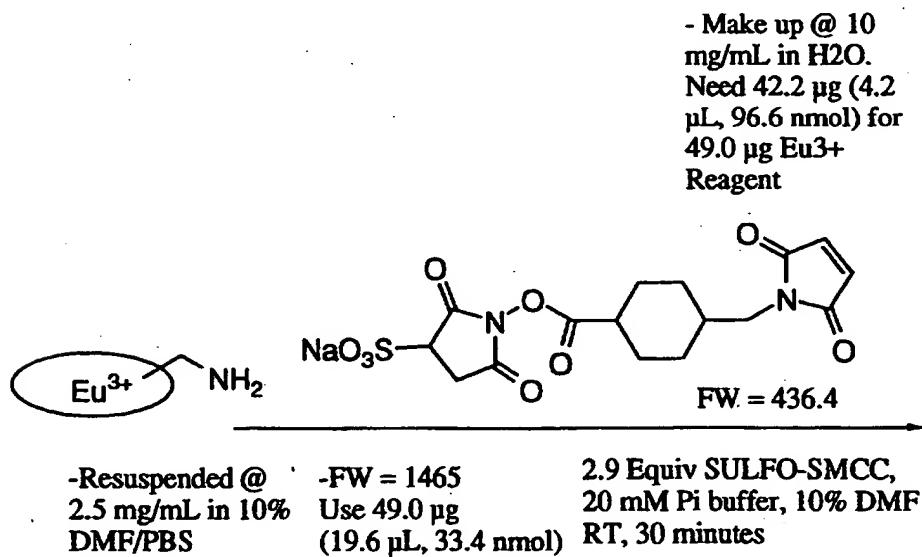
5 occurred in the presence of the agonist AD5075. Although it was clear that FRET was occurring, the signal-noise ratio was small. In experiment 2 of Table 1, HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid, 100 mM, pH 7.0) containing 0.05% NP40 (Nonidet P-40) was used instead of PBS and an improved signal-noise ratio was

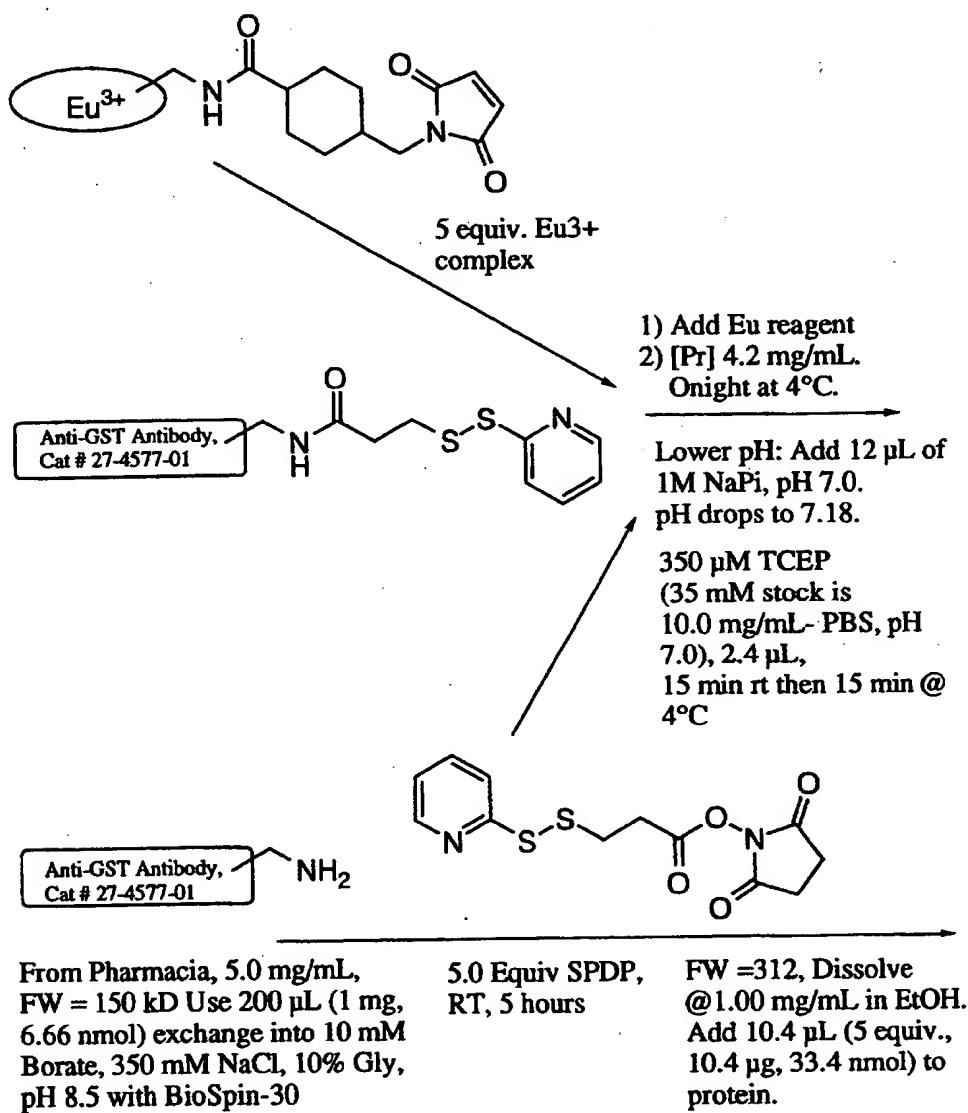
10 obtained.

In order to get an even better signal-noise ratio, the above-described format was modified slightly for experiment 3. In experiment 3, SA-XL665 (500 nM), biotin-labeled hPPAR γ 1LBD (100 nM), GST-hCBP1-113, and Eu3+K labeled anti-GST antibody (2.5 μ L) were incubated

15 in the presence or absence of AD5075 (1 μ M) in HEPES buffer containing 0.05% NP40. A two-fold signal- noise ratio was obtained. Figure 3 shows a schematic of the format used in experiment 3.

The anti-GST antibody was a goat antibody to GST from Pharmacia (catalogue number 27-4577-01) that was labeled with Eu3+K according to the procedure summarized below.





To further improve the signal to noise ratio, a series of experiments were conducted. Experiment 4 of Table 1 exemplifies results obtained from those efforts. cDNA encoding a longer fragment of hCBP was cloned and expressed to get hCBP1-453. hCBP1-453 was biotinylated. Biotin-labeled hCBP1-453 (25 nM), SA-XL665 (100 nM), GST-hPPAR γ 1LBD (1 nM), and Eu3+-K-labeled anti-GST antibody (2 nM) were mixed together in the presence or absence of 1 μ M AD5075. The detergent was changed from 0.05% NP40 to 0.5% CHAPS (3-[{3-cholamidopropyl}dimethyl-ammonio]-1-propanesulfonate). A three- to

5 results obtained from those efforts. cDNA encoding a longer fragment of hCBP was cloned and expressed to get hCBP1-453. hCBP1-453 was biotinylated. Biotin-labeled hCBP1-453 (25 nM), SA-XL665 (100 nM), GST-hPPAR γ 1LBD (1 nM), and Eu3+-K-labeled anti-GST antibody (2 nM) were mixed together in the presence or absence of 1 μ M AD5075. The detergent was changed from 0.05% NP40 to 0.5% CHAPS (3-[{3-cholamidopropyl}dimethyl-ammonio]-1-propanesulfonate). A three- to

10 detergent was changed from 0.05% NP40 to 0.5% CHAPS (3-[{3-cholamidopropyl}dimethyl-ammonio]-1-propanesulfonate). A three- to

four-fold signal-noise ratio was obtained. Figure 4 shows the strategy used for experiment 4 and similar experiments.

- The correlation between results from the above-described assays and previously reported results from *in vitro* binding and transcriptional activation assays of selected antidiabetic insulin sensitizers that are known to be PPAR γ agonists (Elbrecht et al., 1996, Biochem Biophys Res Comm 224:431-437) was analyzed by titrating those known PPAR γ agonists in the assays described above and comparing EC50s so obtained with previously described values for potency in binding or transcriptional activation assays for the known agonists. The results are shown in Figure 5. From Figure 5, the following EC50s can be derived:

AD5075 = 8 nM
BRL49653 = 53 nM
15 Troglitazone = 646 nM
Pioglitazone = 890 nM.

These EC50s generated in the above-described assays are in close agreement with those generated by *in vitro* binding and transcriptional activation studies (Elbrecht et al., 1996, Biochem Biophys Res Comm 224:431-437).

The above-described assay can also be used to characterize the interaction between nuclear receptors with co-activators as, e.g., by determining the binding constant for that interaction. Figure 6 shows an example of such an application. Saturating amounts of PPAR γ 25 agonist (10 μ M BRL49653) were used. Increasing concentrations of non-biotinylated hCBP1-453 were used to titrate away biotin-hCBP-PPAR γ LBD complex and decrease the fluorescence energy transfer. A Kd of 300 nM for the interaction between hCBP1-453 and PPAR γ LBD can be derived from the results illustrated in Figure 6 and this Kd (300 30 nM) is a measurement of the affinity between CBP and PPAR γ .

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such 35 modifications are intended to fall within the scope of the appended claims.

**Various publications are cited herein, the disclosures of
which are incorporated by reference in their entireties.**

WHAT IS CLAIMED:

1. A method of identifying an agonist of a nuclear receptor that comprises providing:
 - (a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;
 - (b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent; and
 - (c) a substance suspected of being an agonist of the nuclear receptor;10 under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, will occur; and
 - (d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents; where the occurrence of FRET indicates that the substance is an agonist of the nuclear receptor.
- 20 2. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, LXR, and FXR.
- 25 3. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a full-length nuclear receptor, a ligand binding domain of a nuclear receptor, and an AF-2 site of a nuclear receptor.
- 30 4. The method of claim 1 where the nuclear receptor or ligand binding domain thereof comprises an AF-2 site of a nuclear receptor.
- 35 5. The method of claim 1 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a

full-length PPAR, a ligand binding domain of a PPAR, and amino acid residues 176-478 of human PPAR γ 1.

6. The method of claim 1 where the nuclear receptor or
5 ligand binding domain thereof is selected from the group consisting of PPAR α , PPAR γ 1, PPAR γ 2, and PPAR δ .

7. The method of claim 1 where the nuclear receptor or
ligand binding domain thereof comprises a ligand binding domain
10 selected from the group consisting of amino acids 143-462 of human RAR α , amino acids 122-410 of rat T₃R α .1, amino acids 227-463 of mouse RXR γ , and amino acids 251-595 of human ER.

8. The method of claim 1 where CBP, p300, or other
15 nuclear receptor co-activator, or a binding portion thereof is selected from the group consisting of full-length human CBP, full-length mouse CBP, amino acid residues 1-113 of human CBP, and amino acid residues 1-453 of human CBP.

20 9. The method of claim 1 where the first fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu³⁺K).

25 10. The method of claim 1 where the second fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu³⁺K).

11. A method of identifying an agonist of a nuclear receptor that comprises providing:

30 (a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;
(b) a binding portion of a nuclear receptor co-activator, where the binding portion contains the amino acid sequence LXXLL, and where the binding portion is labeled with a second fluorescent
35 reagent; and

- (c) a substance suspected of being an agonist of the nuclear receptor;
under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand
5 binding domain thereof and the binding portion of the nuclear receptor co-activator will take place; and
(d) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents;
where the occurrence of FRET indicates that the substance
10 is an agonist of the nuclear receptor.

12. The method of claim 11 where the binding portion of a nuclear receptor co-activator is selected from the group consisting of human RIP-140, human SRC-1, mouse TIF-2, human or mouse CBP,
15 human or mouse p300, mouse TIF-1, and human TRIP proteins.

13. A method of identifying an agonist of a nuclear receptor that comprises providing:
(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;
(b) a polypeptide containing the amino acid sequence LXXLL where the polypeptide is labeled with a second fluorescent reagent; and
25 (c) a substance suspected of being an agonist of the nuclear receptor;
under conditions such that, if the substance is an agonist of the nuclear receptor, binding between the nuclear receptor or ligand binding domain thereof and the polypeptide will take place; and
(d) measuring fluorescent resonance energy transfer (FRET) between the first and second fluorescent reagents;
where the occurrence of FRET indicates that the substance
30 is an agonist of the nuclear receptor.

14. A method for identifying an antagonist of a nuclear receptor that comprises providing:

(a) a nuclear receptor or ligand binding domain thereof labeled with a first fluorescent reagent;

(b) CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a second fluorescent reagent;

5 (c) an agonist of the nuclear receptor; and

(d) a substance suspected of being an antagonist of the nuclear receptor;

under conditions such that, in the absence of the substance, binding between the nuclear receptor or ligand binding domain thereof

10 and CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof will occur; and

(e) measuring fluorescence resonance energy transfer (FRET) between the first and second fluorescent reagents when the substance is present and measuring FRET between the first and second

15 fluorescent reagents when the substance is absent;

where the a decrease in FRET when the substance is present indicates that the substance is an antagonist of the nuclear receptor.

20 15. The method of claim 14 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of steroid receptors, thyroid hormone receptors, retinoic acid receptors, peroxisome proliferator-activated receptors, retinoid X receptors, glucocorticoid receptors, vitamin D receptors, LXR, and FXR.

25

16. The method of claim 14 where the nuclear receptor or ligand binding domain thereof is selected from the group consisting of a full-length nuclear receptor, a ligand binding domain of a nuclear receptor, and an AF-2 site of a nuclear receptor.

30

17. The method of claim 14 where the nuclear receptor or ligand binding domain thereof comprises an AF-2 site of a nuclear receptor.

35

18. The method of claim 14 wh re th nuclear receptor or ligand binding domain thereof is selected from the group consisting of a

full-length PPAR, a ligand binding domain of a PPAR, and amino acid residues 176-478 of human PPAR γ 1.

19. The method of claim 14 where the nuclear receptor or
5 ligand binding domain thereof is selected from the group consisting of
PPAR α , PPAR γ 1, PPAR γ 2, and PPAR δ .

20. The method of claim 14 where the nuclear receptor or
ligand binding domain thereof comprises a ligand binding domain
10 selected from the group consisting of amino acids 143-462 of human
RAR α , amino acids 122-410 of rat T₃R α 1, amino acids 227-463 of mouse
RXR γ , and amino acids 251-595 of human ER.

21. The method of claim 14 where CBP, p300, or other
15 nuclear receptor co-activator, or a binding portion thereof is selected
from the group consisting of full-length CBP, amino acid residues 1-113
of human CBP, and amino acid residues 1-453 of human CBP.

22. The method of claim 14 where the first fluorescent
20 reagent is selected from the group consisting of XL665 and Europium
cryptate (Eu³⁺K).

23. The method of claim 14 where the second fluorescent
reagent is selected from the group consisting of XL665 and Europium
25 cryptate (Eu³⁺K).

24. A nuclear receptor or ligand binding domain thereof
labeled with a fluorescent reagent.

30 25. The nuclear receptor or ligand binding domain
thereof of claim 24 where the nuclear receptor or ligand binding domain
thereof is selected from the group consisting of PPAR α , PPAR γ 1,
PPAR γ 2, PPAR δ , a ligand binding domain of PPAR α , PPAR γ 1, PPAR γ 2,
or PPAR δ , and amino acid residues 176-478 of human PPAR γ 1 and the
35 fluorescent reagent is selected from the group consisting of XL665 and
Europium cryptate (Eu³⁺K).

26. CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, labeled with a fluorescent reagent.

5 27. The CBP, p300, or other nuclear receptor co-activator, or a binding portion thereof, of claim 26 where the fluorescent reagent is selected from the group consisting of XL665 and Europium cryptate (Eu³⁺K).

1/11

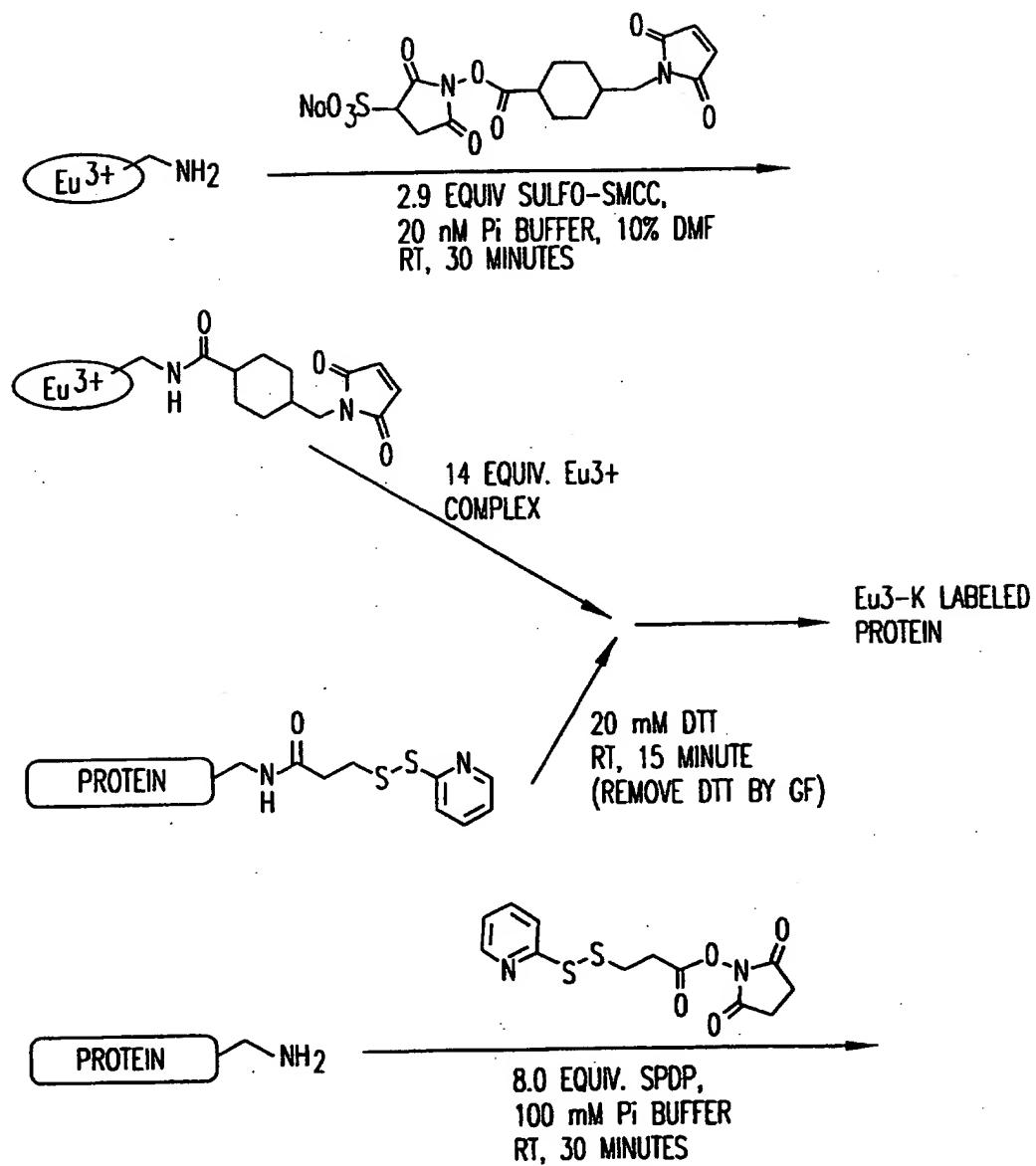


FIG. 1

2/11

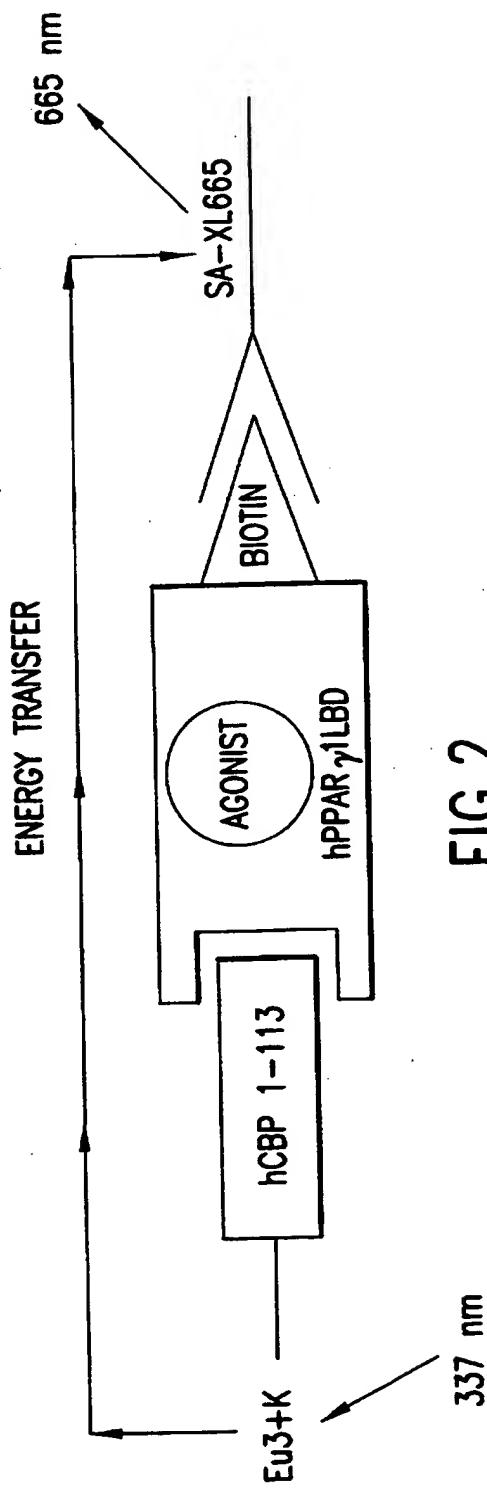


FIG. 2

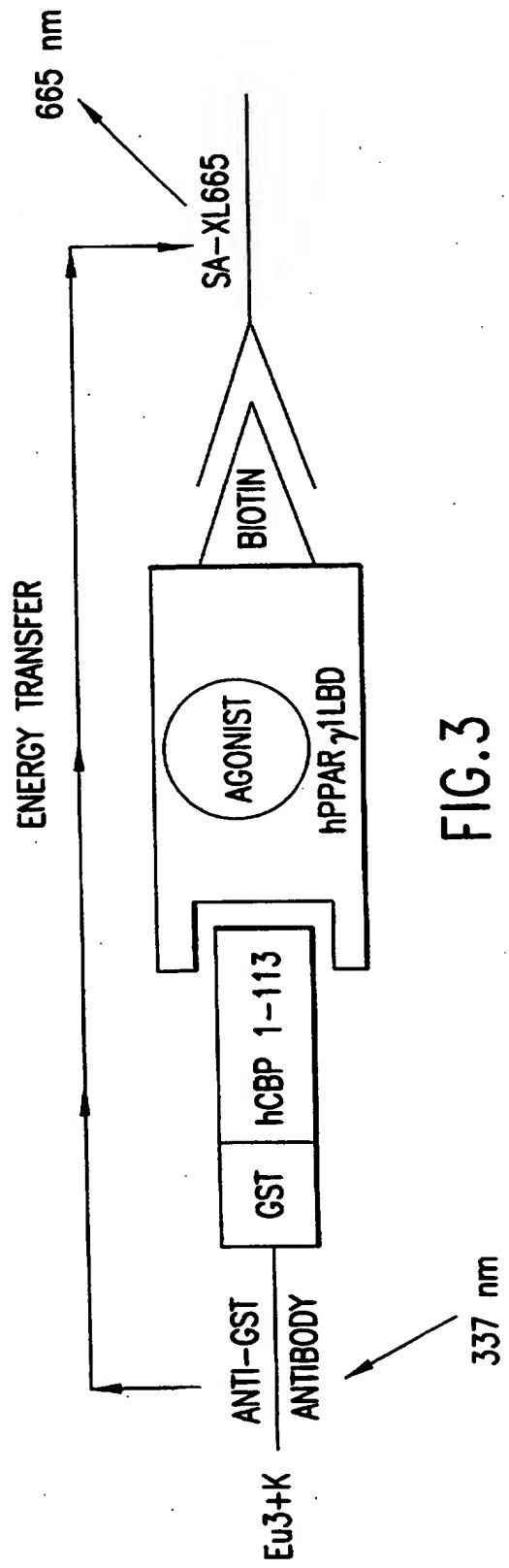


FIG. 3

3/11

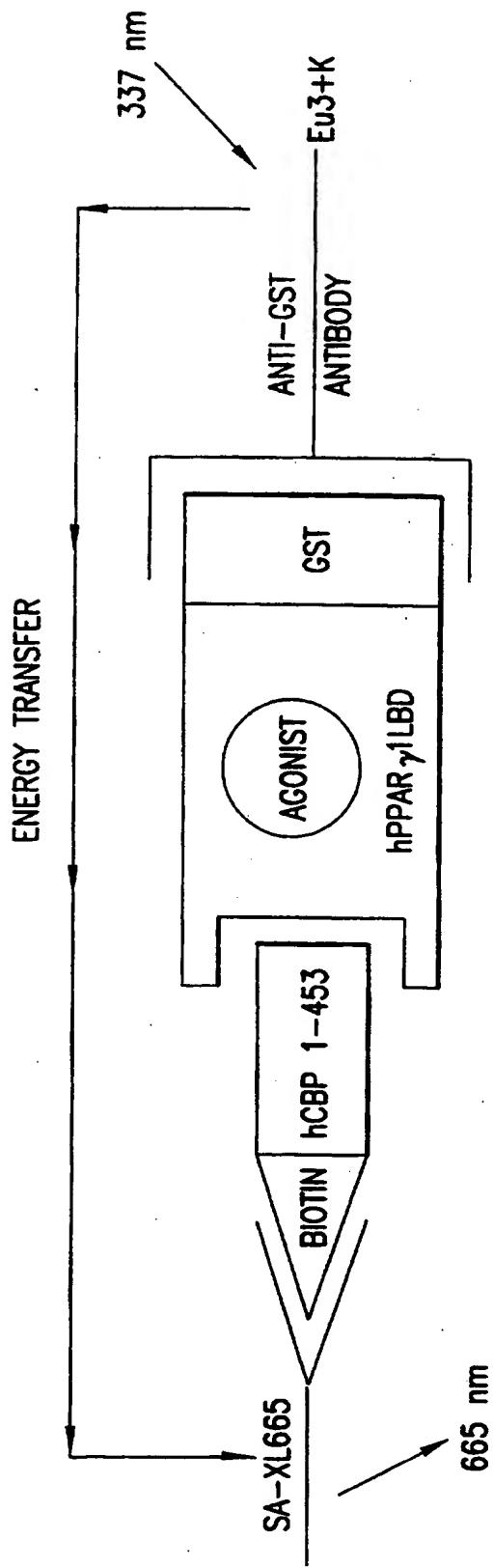


FIG. 4

4/11

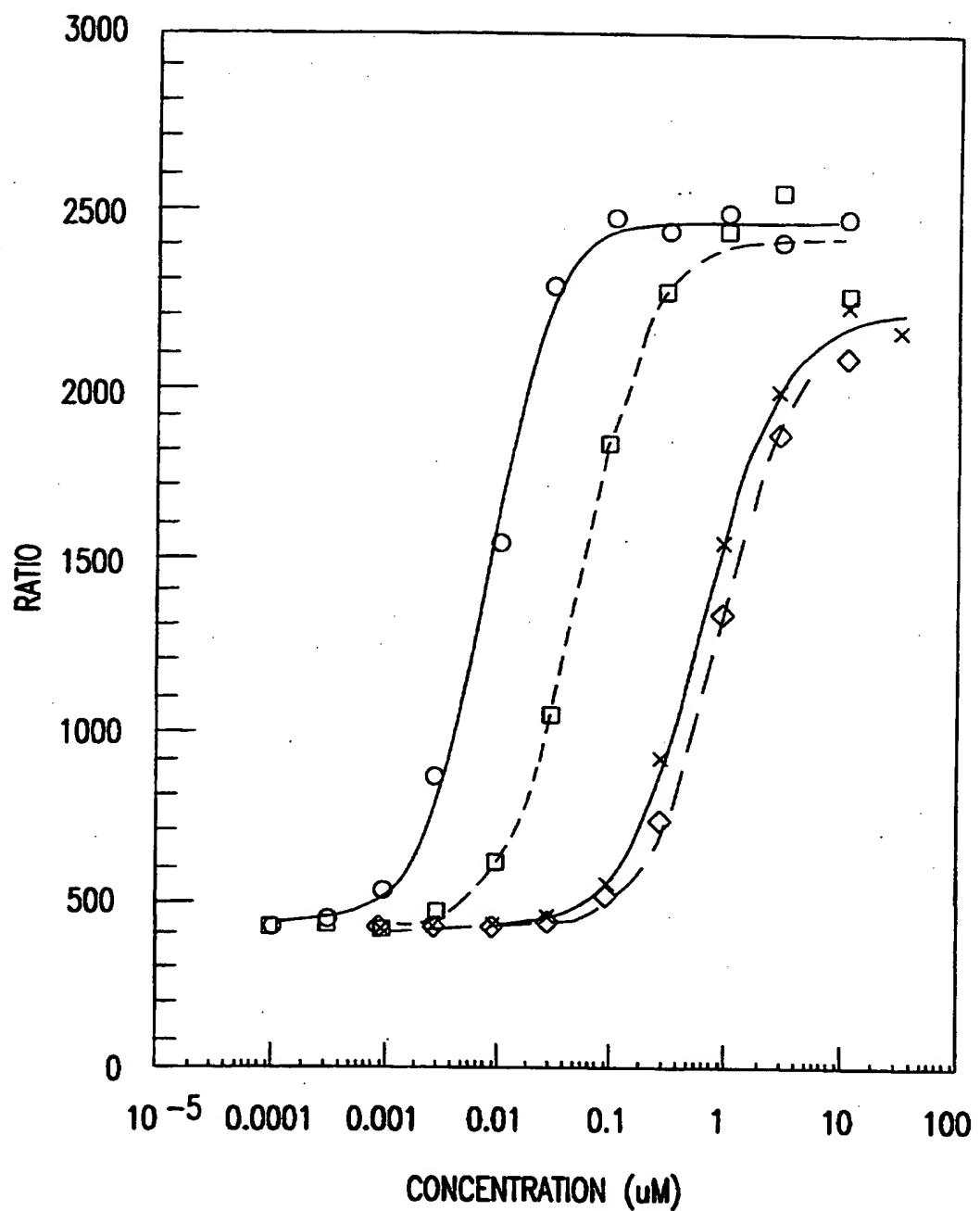


FIG.5

5/11

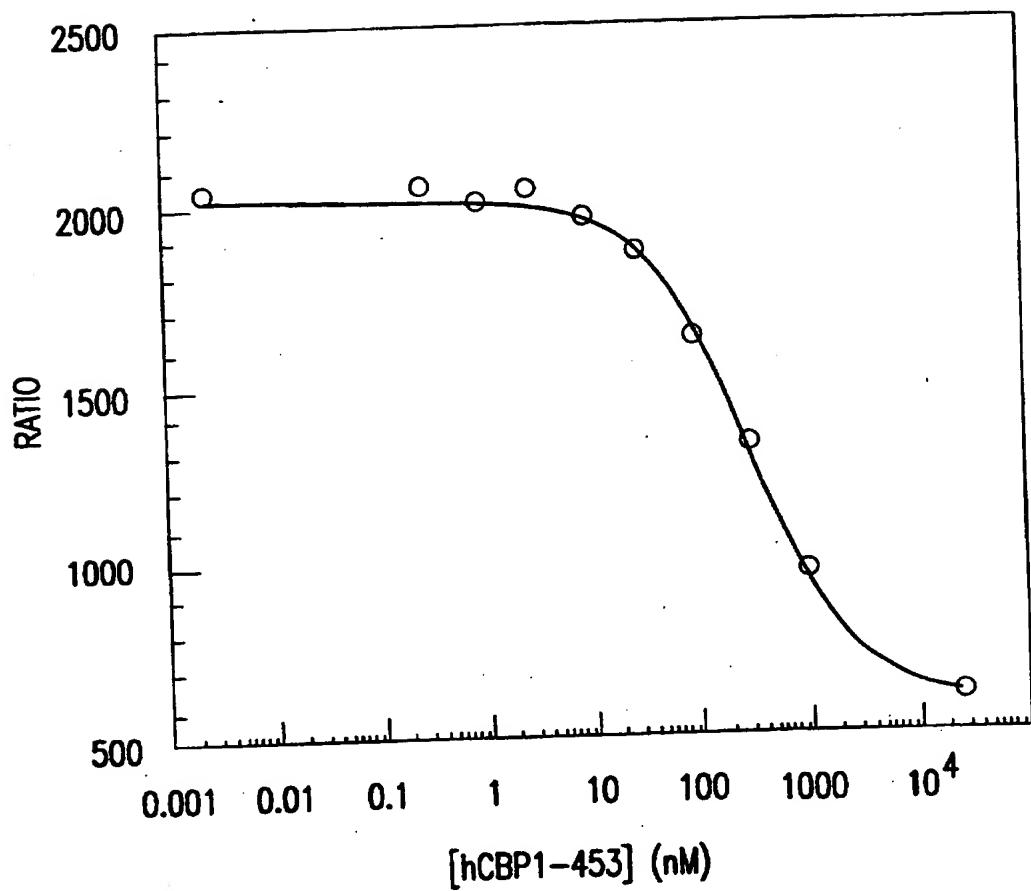


FIG.6

6/11

1 MAENLLDGPPNPKRAKLSSPGFSANDSTDFGSLFDLENDLPDELIPNGGELGLLNSGNLV
61 PDAASKHKQLSELLRGGSGSSINPGIGNVSASSPVQQGLGGQAQGQPNSANMASLSAMGK
121 SPLSQGDSSAPSPLPKQAASSTSGPTPAASQALNPQAQKQVGLATSSPATSGTGPICMNAN
181 FNQTHPGLLNSNSGHSLINQASQGQAQVMNGSLGAAGRGRGAGMPYPTPAMQGASSVLA
241 ETLTQVSPQMTGHAGLNTAQAGGMKGITGNTSPFGQPFQSQAGGQPMGATGVNPQLASK
301 QSMVNSLPTFPTDIKNTSVTNVPNMSQMOTSVGIVPTQAIATGPTADPEKRKLIQQQLVL
361 LLHAHKCQRREQANGEVRACSLPHCRTMKNVLNHMTHCQAGKACQ

FIG. 7A

1 cgagccccga ccccccgtccg ggccctcgcc ggccgcgcgg cccgtgcccc gggctgtttt
61 cccgagcagg taaaaatggc tgagaacttg ctggacggac cgcccaaccc caaaagagcc
121 aaactcagct cgccccggttt ctcggcgaat gacagcacag attttggatc attgtttgac
181 ttggaaaatg attttcctga tgagctgata cccaatggag gagaatttagg ccttttaaac
241 agtggaaacc ttgttccaga tgctgcttcc aaacataaac aactgtcgga gtttctacga
301 ggaggcagcg gctctagtat caaccagga ataggaaatg tgagcgccag cagccccgtg
361 cagcagggcc tgggtggcca ggctcaaggg cagccgaaca gtgctaacat ggccagcctc
421 agtgccatgg gcaagagccc tctgagccag ggagattttt cagccccag cttgcctaaa
481 caggcagcca gcacctctgg gcccacccccc gctgcctccc aagcactgaa tccgcaagca
541 caaaagcaag tggggctggc gactagcagc cctgccacgt cacagactgg acctggatc
601 tgcataatg ctaactttaa ccagacccac ccaggcctcc tcaatagtaa ctctggccat
661 agcttaatta atcaggcttc acaagggcag ggcgaatgca tgaatggatc tcttggggct
721 gctggcagag gaagggggc tggaatgccc taccctactc cagccatgca gggcgccctg
781 agcagcgtgc tggctgagac cctaacgcag gtttccccgc aaatgactgg tcacgcggga
841 ctgaacacccg cacaggcagg aggcatggcc aagatggaa taactggaa cacaagtcca
901 tttggacagc ctttagtca agctggaggg cagccaatgg gagccactgg agtgaacccc
961 cagttagcca gcaaacagag catggtcaac agtttgccta cttccctac agatataag
1021 aataacttcag tcaccaacgt gccaaatatg tctcagatgc aaacatcagt gggaaattgt
1081 cccacacaag caattgcaac aggccccact gcagatcctg aaaaacgc当地 actgatacag
1141 cagcagctgg ttctactgct tcatgctcat aagtgtcaga gacgagagca agcaaacgg
1201 gaggttcggg cctgctcgct cccgcattgt cgaaccatga aaaacgtttt gaatcacatg
1261 acgcattgtc aggctggaa agcctgccaa

FIG. 7B

7/11

1 MVDTESPCPLSPLAEGDLESPLSEEFLOEMGNIQEISQSIGEDSSGSFGFTEYQYLGSC
 61 PGSDGSVITDTLSASSPSSVTYPVPGSVDESPSGALNIECRICGDKASGYHYGVHACE
 121 GCKGFFRRTIRLKLVDKCDRSCKIQKKNRNKQCYCRFHKCLSGMSHNAIRFGRMPRSE
 181 KAKLKAEILTCEHDIEDSETADLKS LAKRIYEAYLKNFMNKVKARVILSGKASNNPPFV
 241 IHDMETLCMAEKTIVAKLVANGIQNKVEVRFHCQCCTS VETVTELTEFAKAIPAFANL
 301 DLNDQVTLLKYGVYEAIFAMLSSVMNKDGMLVAYGNGFITREFLKSURKPFCDIMEPKFD
 361 FAMKFNALELDDSDISLFVAIIICCGDRPGLLNVGHIEKMQEIVHVURLHLQSNHPDDI
 421 FLPKLLQKMADLRQLVTEHAQLVQIIKKTESDAALHPLLQEYRDMY

FIG.8A

1 ggcccaggct gaagctcagg gccctgtctg ctctgtggac tcaacagttt gtggcaagac
 61 aagctcagaa ctgagaagct gtcaccacag ttctggaggc tgggaagtcc aagatcaaag
 121 tgccagcaga ttcatgtca tgtgaggacg tgcttcctgc ttcatagata agatgtac
 181 ggagctcggc ggcacaacca gcaccatctg gtcgcgatgg tggacacgg aagcccactc
 241 tgccccctct cccccactcgaa ggccggcgat cttagagagcc cgttatctga agatccctg
 301 caagaaatgg gaaacatcca agagattcg caatccatcg gcgaggatag ttcttggaaagc
 361 tttggctta cggaaatcca gtatttagga agctgtcctg gctcagatgg ctcggtcatc
 421 acggacacgc tttcaccacg ttccgagcccc tcctcggtgaa cttatcctgt ggtccccggc
 481 agcgtggacg agtctcccg tggagcattt aacatcgaat gtagaatctg cggggacaag
 541 gcctcaggct atcattacgg agtccacgcg tgtgaaggct gcaaggcctt ctttcggcga
 601 acgattcgac tcaagcttgt gtatgacaag tgccgaccga gctgcaagat ccagaaaaaag
 661 aacagaaaaca aatgccagta ttgtcgattt cacaagtgc tttctgtcgg gatgtcacac
 721 aacgcgattt gttttggacg aatgccaaga tctgagaaag caaaaactgaa agcagaaaatt
 781 cttacctgtg aacatgacat agaagattct gaaactgcag atctcaaatc tctggccaag
 841 agaatctacg aggcctactt gaagaacttc aacatgaaaca agtcaaagc ccgggtcattc
 901 ctctcaggaa aggccagtaa caatccaccc tttgtcatac atgatatgg gacactgtgt
 961 atggctgaga agacgcttgt ggccaaatgg gtggccaaatg gcatccagaa caaggaggtg
 1021 gaggtccgca tcttcactg ctgcaggatgc acgtcagtgg agaccgtcac ggagctcacg
 1081 gaattcgcca aggccatccc agcgttcgca aacttggacc tgaacgatca agtgcattt
 1141 ctaaaaatacg gagtttatga ggccatattt gccatgtgt cttctgttatgaaacaaagac
 1201 gggatgtctgg tagcgtatgg aaatgggttt ataactcgatg aattcctaaa aagcctaagg
 1261 aaaccgttct gtgatatcat ggaacccaaatgg tttgattttt ccatgaagtt caatgcactg
 1321 gaactggatg acagtgtat cttccctttt gtggctgcta tcatttgctg tggagatcgt
 1381 cctggccttc taaacgttagg acacattgaa aaaatgcagg agggattttt acatgtgctc
 1441 agactccacc tgcagagcaa ccacccggac gatatcttc tcttccaaa acttctcaa
 1501 aaaatggcag acctccggca gctggtgacg gagcatgcgc agctggtgca gatcatcaag
 1561 aagacggagt cggatgctgc gctgcacccg ctactgcagg agatctacag ggacatgtac
 1621 tgagttcctt cagatcagcc acaccattttc caggagttct gaagctgaca gcaactacaaa
 1681 ggagacgggg gaggcagcag attttgcaca aatatccacc acttaaccc tagagcttgg
 1741 acagtctgag ctgttagttaa ccggcatattt attccatatc tttgttttaa ccagtacttc
 1801 taagagcata gaactcaaattt gctgggggag gtggctaatc tcaggactgg gaag

FIG.8B

8/11

1 MTMVDTEIAFWPTNFGISSVDSL SVMEDHSHSF DIKPFTTVDSSISTPHYEDIPFRTDP
 61 VVADYKYDLKLQEQYQSAIKVEPASPPYYSEKTQLYNKPHEEPSNSLMAIECRVCGDKASG
 121 FHYGVHACEGCKGFFRRTIRLKLIYDRCDLNCRIHKKSRNKCQYCRFQKCLAVGM SHNAI
 181 RFGRIAQAEEKEKLLAEISSDIDQLNPESADLRQALAKLYDSYIKSFPLTKAKARAILTG
 241 KTTDKSPFVIYDMNSLMMGEDKIKFKHITPLQEKSKEVAIRIFQGCQFRSVEAVQEITEY
 301 AKSIPGFVNLDLNDQVTLLKYGVHEIIYTMLASLMNKDGVLISEGQGFMTREFLKSLRKP
 361 FGDFMEPKFEFAVKFN ALELDDSDLAIFI AVIILSGDRPGLLNVKPIEDIQDNLLQALEL
 421 QLKLNHPESSQUAKLLQKMTDLRQIVTEHVQLLQVIKKTETDMSLHPLLQEIYKDLY

FIG.9A

1 ccgacattac cccaggcggc cttgacgtt gtttcgtt caggagacag caccatggg
 61 ggttctctt gaggatggaa attcccgagc ccgagccgc gcccgcctt ggggggctt
 121 ggtcggcctc gaggacaccg gagaggggcg ccacgcgc gttggccgc agaatgaccat
 181 ggttgacaca gagatcgcat tctggccac caactttggg atcagctccg tggatctctc
 241 cgtaatggaa gaccactccc actccttga tatcaagccc ttcaactactg ttgacttctc
 301 cagcatttct actccacatt acgaagacat tccattcaca agaacagatc cagtgggtgc
 361 agattacaag tatgacctga aacttcaaga gtaccaaagt gcaatcaaag tggagcctgc
 421 atctccacat tattattctg agaagactca gctctacaat aagcctcatg aagagccttc
 481 caactccctc atggcaattt aatgtcgtgt ctgtggagat aaagcttctg gatttcaacta
 541 tggagttcat gtttgtgaag gatgcaaggg ttcttccgg agaacaatca gattgaagct
 601 tatctatgac agatgtgatc ttaactgtcg gatccacaaa aaaagttagaa ataaatgtca
 661 gtactgtcg tttcagaaat gccttgcagt gggatgtct cataatgcca tcagggttgg
 721 gcggatcgca caggccgaga aggagaagct gttggccgg atctccagt atatcgacca
 781 gctgaatcca gagtccgctg acctccgtca ggccctggca aaacatttgc atgactcata
 841 cataaagtcc ttcccgtga ccaaagcaaa ggcgaggcg atcttgcacag gaaagacaac
 901 agacaaatca ccattcgta tctatgacat gaattcccta atgatgggag aagataaaat
 961 caagttaaaa cacatcaccc ccctgcagga gcagacaaa gaggtggcca tccgcacatctt
 1021 tcagggctgc cagttcgct ccgtggaggc tgtgcaggag atcacagagt atgcacaaag
 1081 cattccttgtt tttgtaaatc ttgacttgaa cgaccaagta actctcctca aatatggagt
 1141 ccacgagatc atttacacaa tgctggcctc ttgtatgaaat aaagatgggg ttctcatatc
 1201 cgagggccaa ggcttcatga caagggagtt tctaaagagc ctgcggaaagc cttttgggtga
 1261 ctttatggag cccaa gtttgcgtgt gaagttcaat gcactggaaat tagatgacag
 1321 cgacttggca atatttattt ctgtcattat tctcagtggc gaccgcccag gtttgcgtaa
 1381 tgtgaagccc attgaagaca ttcaagacaa cctgtacaa gccctggagc tccagctgaa
 1441 gctgaaccac cctgagtcct cacagctgtt tgccaa gctc cccagaaaa tgacagacat
 1501 cagacagatt gtcacggAAC acgtgcagct actgcaggat atcaagaaga cggagacaga
 1561 catgagtctt cacccgtcc tgcaggagat ctacaaggac ttgtactagc agagagtcc
 1621 gagccactgc caacattcc cttttccag ttgcactatt ctgaggaaa atctgaccat
 1681 aagaaaattt ctgtgaaaaa gcgtttaaa aagaaaaggg tttagaatat gatctatattt
 1741 atgcattttt tttataaaga cacattaca atttacttt aatattaaaa attaccatatt
 1801 tatgaaattt c

FIG.9B

9/11

1 MEOPQEEAPEVREEEEKEEVAEAEGAPELNGGPQHALPSSSYTDLSSRSPSLLDQLQM
61 GCPGASCGLNMECRVCGDKASGFHYGVHACEGCKGFFRRTIRMKLEYKCERSCKIQKK
121 NRNCQYCRFQKCLALGMSHNAIRFGRMPEAEKRKLVAGLTANEQSQYNPQADLKAFSK
181 HIYNAYLKNFNMTKKARSILTGKASHTAPFVIHDIETLWQAEGKLWKQLVNGLPPYKE
241 ISVHVFYRCQCTTVETVRELTEFAKSIPSFSFLNNDQVTLLKGVHEAIFAMLASIVNK
301 DGLLVANGSGFTREFLRSLRKPFSDIIEPKFEFAVKFNALELDDSDLALFIAAIILCGD
361 RPGLMNVRVEAIQDTILRALEFHLOQANHPDAQYLFPKLLQKMADLRQLVTEHAQMMQRI
421 KKTETETSLHPLLQEIYKDMY

FIG.10A

10/11

1 gaattctgcg gaggcctgcgg gacggcgccg ggttggccc taggcagccg ggacagtgtt
61 gtacagtgtt ttgggcatgc acgtgatact cacacagtgg cttctgctca ccaacagatg
121 aagacagatg caccAACAGAG ggtctggaaat ggtctggagt ggtctggaaa gcagggtcag
181 ataccCCTGG AAAACTGAAG CCCGTGGAGC aatgatctc acaggactgc ttcaaggctg
241 atgggaacca ccctgttagag gtccatctgc gttcagaccc agacgatgcc agagctatga
301 ctgggcctgc aggtgtggcg ccgaggggag atcagccatg gagcagccac aggaggaagc
361 ccctgaggTC CGGGAAGAGG aggagaaaaga ggaagtgcA gaggcagaag gagccccaga
421 gctcaatggg ggaccacagc atgcacttcc ttccagcagc tacacagacc tctccggag
481 ctccctcgcca ccctcaCTGC tggaccaact gcagatggc tgtacgggg cctcatgcgg
541 cagcctcaac atggagtGCC gggtgtgcgg ggacaaggca tcggcttcc actacgggt
601 tcatgcatgt gaggggtgca agggcttctt ccgtcgtacg atccgcatga agctggagta
661 cgagaagtgt gagcgcagct gcaagattca gaagaagaac cgcaacaagt gccagtactg
721 ccgcttccag aagtgcctgg cactggcat gtcacacaac gctatccgtt ttggtcggat
781 gccggaggct gagaagagga agctgggtgc agggctgact gcaaacgagg ggagccagta
841 caaccacag gtggccgacc tgaaggcctt ctccaagcac atctacaatg cctacctgaa
901 aaacttcaac atgaccaaaa agaaggccc cagcatcctc accggcaaag ccagccacac
961 ggcgccctt gtatccacg acatcgagac attgtggcag gcagagaagg ggctgggtg
1021 gaagcagttg gtatggcc tgcctcccta caaggagatc agcgtgcacg tcttctaccg
1081 ctgcccagtgc accacagtgg agaccgtgcg ggagctcact gagttcgcca agagcatccc
1141 cagcttcagc agccttcc tcaacgacca gtttaccctt ctcaagtatg gcgtgcacga
1201 ggccttc gccatgtgg cctctatcgta caacaaggac gggctgtgg tagccaacgg
1261 cagttggctt gtcacccgtg agttcgtgcg cagcctccgc aaaccctca gtgatatcat
1321 tgagcctaag tttgaattt ctgtcaagtt caacgcctg gaacttgatg acagtgaccc
1381 ggccttattt attgcggca tcattctgtg tggagaccgg ccaggcctca tgaacgttcc
1441 acgggtggag gctatccagg acaccatcct gcgtgcctc gaattccacc tgcaggccaa
1501 ccaccctgat gcccagtacc tctcccaa gctgtgcag aagatggctg acctgcggca
1561 actggtcacc gaggcacccc agatgtgcg gcggatcaag aagaccgaaa ccpagaccc
1621 gctgcacct ctgctccagg agatctacaa ggacatgtac taacggcggc acccaggcct
1681 ccctgcagac tccaatgggg ccagcaactgg aggggcccac ccacatgact tttccattga
1741 ccagctctt ccctgtctt gttgtctccc tcttctcgat ttccctttt tttctaatt
1801 cctgttgc ttttcttcc tttctgttagg tttctctt ccctctccc ttctccctt
1861 ccctccctt ctcttccta tccccacgtc tgtcctcctt tcttattctg tgagatgttt
1921 tgtattattt caccagcagc atagaacagg acctctgctt ttgcacaccc tttcccccagg
1981 agcagaagag agtgggcctg ccctctgccc catattgc a cctgcaggct taggtcctca
2041 cttctgtctc ctgtcttcag agcaaaaagac ttgagccatc caaagaaaaca ctaagcttc
2101 tgggcctggg ttccaggaa ggctaagcat ggcctggact gactgcagcc ccctatagtc
2161 atggggtccc tgctgcaaag gacagtggca gaccccccgt gtagagccgatgcctccc
2221 caagactgtc attgcccctc cgatcgttag ggcacccact gacccaaatga tcctctccag
2281 cagcacaccc t cagcccaact gacacccagt gtccttccat ctccacactg gtttgcagg
2341 ccaatgttgc tgatggccccc tccagcacac acacataagc actgaaatca cttaacctgc
2401 agcaccatg cacccctt ccctccctga ggcaggttag aacccagaga gagggcctg

FIG. 10B

11/11

2461 caggtgagca ggcaggcgtg ggccaggtct ccggggaggc aggggtcctg caggtcctgg
2521 tgggtcagcc cagcacctcg cccagtggga gcttccccc ataaacttag cctgttcatt
2581 ctgtatgtcca ttgtcccaa tagctctact gccctccct tccccttac tcagcccagc
2641 tggccaccta gaagtctccc tgacacagcct ctatgtccg gggacattgt gggaccagtc
2701 ccacaccgct ggtccctgccc ctccctgtct cccaggttga ggtgcgtca cctcagagca
2761 gggccaaagc acagctggc atgcatgtc tgagcggcgc agagccctcc aggccctgcag
2821 gggcaagggg ctggctggag ttcagagca cagaggttagg agaactgggg ttcaagccca
2881 ggcttcctgg gtcctgcctg gtcctccctc ccaaggagcc attctatgt actctgggtg
2941 gaagtgccta gcccctgcct gacggnnnnn nngatcaactc tctgctggca ggattcttcc
3001 cgctccccac ctacccagct gatgggggtt ggggtgcctc tttcagccaa ggctatgaag
3061 ggacagctgc tgggaccac ctccccccctt ccccgccac atgccgcgtc cctgccccca
3121 cccgggtctg gtgctgagga tacagctttt ctcagtgtct gaacaatctc caaaattgaa
3181 atgtatattt ttgcttaggag ccccagcttc ctgtttttt aatataaata gtgtacacag
3241 actgacgaaa cttaataaa atggaaatta aatatttaaa aaaaaaagcg gccgcgaatt
3301 c

FIG. 10C

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Merck & Co., Inc.
- (ii) TITLE OF INVENTION: ASSAYS FOR NUCLEAR RECEPTOR AGONISTS AND ANTAGONISTS USING FLUORESCENCE RESONANCE ENERGY TRANSFER

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Merck & Co., Inc.
- (B) STREET: P.O. Box 2000, 126 E. Lincoln Ave.
- (C) CITY: Rahway
- (D) STATE: NJ
- (E) COUNTRY: USA
- (F) ZIP: 07065-0900

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Diskette
- (B) COMPUTER: IBM Compatible
- (C) OPERATING SYSTEM: Windows
- (D) SOFTWARE: FastSEQ for Windows Version 2.0b

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Coppola, Joseph A
- (B) REGISTRATION NUMBER: 38,413
- (C) REFERENCE/DOCKET NUMBER: 20017PCT

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 732-594-6734
- (B) TELEFAX: 732-594-4720
- (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 405 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ala Glu Asn Leu Leu Asp Gly Pro Pro Asn Pro Lys Arg Ala Lys
 1 5 10 15
 Leu Ser Ser Pro Gly Phe Ser Ala Asn Asp Ser Thr Asp Phe Gly Ser
 20 25 30
 Leu Phe Asp Leu Glu Asn Asp Leu Pro Asp Glu Leu Ile Pro Asn Gly
 35 40 45
 Gly Glu Leu Gly Leu Leu Asn Ser Gly Asn Leu Val Pro Asp Ala Ala
 50 55 60
 Ser Lys His Lys Gln Leu Ser Glu Leu Leu Arg Gly Gly Ser Gly Ser
 65 70 75 80
 Ser Ile Asn Pro Gly Ile Gly Asn Val Ser Ala Ser Ser Pro Val Gln
 85 90 95
 Gln Gly Leu Gly Gln Ala Gln Gly Gln Pro Asn Ser Ala Asn Met
 100 105 110
 Ala Ser Leu Ser Ala Met Gly Lys Ser Pro Leu Ser Gln Gly Asp Ser
 115 120 125
 Ser Ala Pro Ser Leu Pro Lys Gln Ala Ala Ser Thr Ser Gly Pro Thr
 130 135 140
 Pro Ala Ala Ser Gln Ala Leu Asn Pro Gln Ala Gln Lys Gln Val Gly
 145 150 155 160
 Leu Ala Thr Ser Ser Pro Ala Thr Ser Gln Thr Gly Pro Gly Ile Cys
 165 170 175
 Met Asn Ala Asn Phe Asn Gln Thr His Pro Gly Leu Leu Asn Ser Asn
 180 185 190
 Ser Gly His Ser Leu Ile Asn Gln Ala Ser Gln Gly Gln Ala Gln Val
 195 200 205
 Met Asn Gly Ser Leu Gly Ala Ala Gly Arg Gly Arg Gly Ala Gly Met
 210 215 220
 Pro Tyr Pro Thr Pro Ala Met Gln Gly Ala Ser Ser Ser Val Leu Ala
 225 230 235 240
 Glu Thr Leu Thr Gln Val Ser Pro Gln Met Thr Gly His Ala Gly Leu
 245 250 255
 Asn Thr Ala Gln Ala Gly Gly Met Ala Lys Met Gly Ile Thr Gly Asn
 260 265 270
 Thr Ser Pro Phe Gly Gln Pro Phe Ser Gln Ala Gly Gly Gln Pro Met
 275 280 285
 Gly Ala Thr Gly Val Asn Pro Gln Leu Ala Ser Lys Gln Ser Met Val
 290 295 300
 Asn Ser Leu Pro Thr Phe Pro Thr Asp Ile Lys Asn Thr Ser Val Thr
 305 310 315 320
 Asn Val Pro Asn Met Ser Gln Met Gln Thr Ser Val Gly Ile Val Pro
 325 330 335
 Thr Gln Ala Ile Ala Thr Gly Pro Thr Ala Asp Pro Glu Lys Arg Lys
 340 345 350
 Leu Ile Gln Gln Leu Val Leu Leu His Ala His Lys Cys Gln
 355 360 365
 Arg Arg Glu Gln Ala Asn Gly Glu Val Arg Ala Cys Ser Leu Pro His
 370 375 380
 Cys Arg Thr Met Lys Asn Val Leu Asn His Met Thr His Cys Gln Ala
 385 390 395 400
 Gly Lys Ala Cys Gln
 405

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1290 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGAGCCCCGA	CCCCCGTCGG	GGCCCTCGCC	GGCCGCGCCG	CCCGTGCCTCG	GGGCTGT	60
CCCGAGCAGG	TGAAAATGCC	TGAGAACTTG	CTGGACGGAC	CGCCCAACCC	CAAAGAGCC	120
AAACTCAGCT	CGCCCGGTTT	CTCGGCGAAT	GACAGCACAG	ATTGGATC	ATGTTTGAC	180
TIGGAAAATG	ATCTTCCCTGA	TGAGCTGATA	CCCAATGGAG	GAGAATTAGG	CCTTTAAAC	240
AGTGGGAACC	TTGTGCGGAGA	TGCTGCTTCC	AAACATAAAC	AACGTGCGGA	GCTTCTACGA	300
GGAGGGCAGCG	GCTCTAGTAT	CAACCCAGGA	ATAGGAAATG	TGAGCGCCAG	CAGCCCCGTG	360
CAGCAGGGCC	TGGGTGGCCA	GGCTCAAGGG	CAGCCGAACA	GTGCTAACAT	GGCCAGCCTC	420
AGTGCCATGG	GCAAGAGCCC	TCTGAGGCC	GGAGATTCTT	CAGCCCCCAG	CCTGCCCTAAA	480
CAGGCAGCCA	GCACCTCTGG	GCCCACCCCC	GCTGCCCTCCC	AAGCAGTGA	TCCGCAAGCA	540
CAAAGCAAG	TGGGGCTGGC	GACTAGCAGC	CCTGCCACCGT	CACAGACTGG	ACCTGGTATC	600
TGCATGAATG	CTAACTTTAA	CCAGACCCAC	CCAGGCCCTCC	TCAATAGTAA	CTCTGGCAT	660
AGCTTAATTA	ATCAGGCTTC	ACAAGGGCAG	GCGCAAGTCA	TGAATGGATC	TCTTGGGGCT	720
GCTGGCAGAG	GAAGGGGGAGC	TGGAATGCCG	TACCCCTACTC	CAGCCATGCA	GGGCGCCTCG	780
AGCAGGGTGC	TGGCTGAGAC	CCTAACCGCAG	GTTCCCCCCG	AAATGACTGG	TCACGGGGAA	840
CTGAACACCG	CACAGGCAGG	AGGCATGGCC	AAGATGGAA	TAACTGGGAA	CACAAGTCCA	900
TTTGGACAGC	CCTTTAGTCA	AGCTGGAGGG	CAGCCAATGG	GAGCCACTGG	AGTGAACCCC	960
CAGTTAGCCA	GCAAAACAGAG	CATGGTCAAC	AGTTTGCCCA	CCTTCCCTAC	AGATATCAAG	1020
AATACTTCAG	TCACCAACGT	GCCAAATATG	TCTCAGATGC	AAACATCAGT	GGGAATTGTA	1080
CCCACACAAG	CAATTGCAAC	AGCCCCCACT	GCAGATCTG	AAAAACGCAA	ACTGATAACAG	1140
CAGCAGCTGG	TTCCTACTGCT	TCATGCTCA	AAGTGTCAAGA	GACAGAGGCA	AGCAAACGGA	1200
GAGGTTCCGG	CCTGCTCGCT	CCCGCATTGT	CGAACCATGA	AAAACGTTT	GAATCACATG	1260
ACGCATTGTC	AGGCTGGAA	AGCCCTGCCAA				1290

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 468 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met	Val	Asp	Thr	Glu	Ser	Pro	Leu	Cys	Pro	Leu	Ser	Pro	Leu	Glu	Ala
1				5					10				15		
Gly	Asp	Leu	Glu	Ser	Pro	Leu	Ser	Glu	Glu	Phe	Leu	Gln	Glu	Met	Gly
					20				25			30			
Asn	Ile	Gln	Glu	Ile	Ser	Gln	Ser	Ile	Gly	Glu	Asp	Ser	Ser	Gly	Ser
					35				40			45			
Phe	Gly	Phe	Thr	Glu	Tyr	Gln	Tyr	Leu	Gly	Ser	Cys	Pro	Gly	Ser	Asp
					50				55			60			

Gly Ser Val Ile Thr Asp Thr Leu Ser Pro Ala Ser Ser Pro Ser Ser
 65 70 75 80
 Val Thr Tyr Pro Val Val Pro Gly Ser Val Asp Glu Ser Pro Ser Gly
 85 90 95
 Ala Leu Asn Ile Glu Cys Arg Ile Cys Gly Asp Lys Ala Ser Gly Tyr
 100 105 110
 His Tyr Gly Val His Ala Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg
 115 120 125
 Thr Ile Arg Leu Lys Leu Val Tyr Asp Lys Cys Asp Arg Ser Cys Lys
 130 135 140
 Ile Gln Lys Lys Asn Arg Asn Lys Cys Gln Tyr Cys Arg Phe His Lys
 145 150 155 160
 Cys Leu Ser Val Gly Met Ser His Asn Ala Ile Arg Phe Gly Arg Met
 165 170 175
 Pro Arg Ser Glu Lys Ala Lys Leu Lys Ala Glu Ile Leu Thr Cys Glu
 180 185 190
 His Asp Ile Glu Asp Ser Glu Thr Ala Asp Leu Lys Ser Leu Ala Lys
 195 200 205
 Arg Ile Tyr Glu Ala Tyr Leu Lys Asn Phe Asn Met Asn Lys Val Lys
 210 215 220
 Ala Arg Val Ile Leu Ser Gly Lys Ala Ser Asn Asn Pro Pro Phe Val
 225 230 235 240
 Ile His Asp Met Glu Thr Leu Cys Met Ala Glu Lys Thr Leu Val Ala
 245 250 255
 Lys Leu Val Ala Asn Gly Ile Gln Asn Lys Glu Val Glu Val Arg Ile
 260 265 270
 Phe His Cys Cys Gln Cys Thr Ser Val Glu Thr Val Thr Glu Leu Thr
 275 280 285
 Glu Phe Ala Lys Ala Ile Pro Ala Phe Ala Asn Leu Asp Leu Asn Asp
 290 295 300
 Gln Val Thr Leu Leu Lys Tyr Gly Val Tyr Glu Ala Ile Phe Ala Met
 305 310 315 320
 Leu Ser Ser Val Met Asn Lys Asp Gly Met Leu Val Ala Tyr Gly Asn
 325 330 335
 Gly Phe Ile Thr Arg Glu Phe Leu Lys Ser Leu Arg Lys Pro Phe Cys
 340 345 350
 Asp Ile Met Glu Pro Lys Phe Asp Phe Ala Met Lys Phe Asn Ala Leu
 355 360 365
 Glu Leu Asp Asp Ser Asp Ile Ser Leu Phe Val Ala Ala Ile Ile Cys
 370 375 380
 Cys Gly Asp Arg Pro Gly Leu Leu Asn Val Gly His Ile Glu Lys Met
 385 390 395 400
 Gln Glu Gly Ile Val His Val Leu Arg Leu His Leu Gln Ser Asn His
 405 410 415
 Pro Asp Asp Ile Phe Leu Phe Pro Lys Leu Leu Gln Lys Met Ala Asp
 420 425 430
 Leu Arg Gln Leu Val Thr Glu His Ala Gln Leu Val Gln Ile Ile Lys
 435 440 445
 Lys Thr Glu Ser Asp Ala Ala Leu His Pro Leu Leu Gln Glu Ile Tyr
 450 455 460
 Arg Asp Met Tyr
 465

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

WO 99/18124

- (A) LENGTH: 1854 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGCCCAGGCT	GAAGCTCAGG	GCCCTGTCTG	CTCTGTGGAC	TCAACAGTTT	GTGGCAAGAC	60
AAGCTCAGAA	CTGAGAAGCT	GTCACCACAG	TTCAGGAGGC	TGGGAAGTTC	AAGATCAAAG	120
TGCCAGCAGA	TTCAGTGTCA	TGTGAGGACG	TGCTTCCTGC	TTCATAGATA	AGAGTAGCTT	180
GGAGCTCGGC	GGCACAAACCA	GCACCACATCTG	GTCGCAGATGG	TGGACACGGA	AAGCCCACTC	240
TGCCCCCTCT	CCCCACTCGA	GGCCGGCGAT	CTAGAGAGCC	CGTTATCTGA	AGAGTTCCCTG	300
CAAGAAATGG	GAAACATCCA	AGAGATTTTG	CAATCCATCG	GCGAGGATAG	TTCTGGAAGC	360
TTTGGCTTA	CGGAATACCA	GTATTTAGGA	AGCTGTCTG	GCTCAGATGG	CTCGGTATC	420
ACGGACACGC	TTTCACCAACG	TTCGAGCCCC	TCCCTCCGTGA	CTTATCTGTG	GGTCCCCGGC	480
ACCGTGGACG	AGTCTCCAG	TGGAGCATTG	AACATCGAAT	GTAGAATCTG	GGGGGACAAG	540
GCCTCAGGCT	ATCATTACGG	AGTCCACCGG	TGTGAAGGCT	GCAAGGGCTT	CTTTCGGCGA	600
ACGATTGAC	TCAAGCTGGT	GTATGACAAG	TGGCAGCGCA	GCTGCAAGAT	CCAGAAAAG	660
AACAGAAACA	AATGCCAGTA	TTGTCGATTT	CACAAGTGCC	TTTCTGTCGG	GATGTCACAC	720
AACCGGATTG	TTTTGGACG	AATGCCAAGA	TCTGAGAAAG	CAAACATGAA	AGCAGAAATT	780
CTTACCTGTG	AAACATGACAT	AGAAGATTCT	GAAACTGCAG	ATCTCAAATC	TCTGGCCAAG	840
AGAATCTACG	AGGGCTACTT	GAAGAACTTC	AAACATGAACA	AGGTCAAAGC	CCGGGTATC	900
CTCTCAGGAA	AGGCCAGTAA	CAATCCACT	TTTGTACATAC	ATGATATGGG	GACACTGTGT	960
ATGGCTGAGA	AGACGCTGGT	GGCCAAGCTG	GTGGCCAATG	GCATCCAGAA	CAAGGAGGTG	1020
GAGGTCCGCA	TCTTTCACTG	CTGCCAGTGC	ACGTCAGTGC	AGACCGTCAC	GGAGCTCACG	1080
GAATTGCCA	AGGCCATCCC	AGCGTTCCA	AACTTGGACC	TGAACGATCA	AGTGACATTG	1140
CTAAAATACG	GAGTTTATGA	GGCCATATTC	GCCATCTGT	CTTCTGTGAT	GAACAAAGAC	1200
GGGATGCTGG	TACCGTATGG	AAATGGGTTT	ATAACTCTGT	AATTCTAAA	AAGCCTAAGG	1260
AAACCGTCT	GTGATATCAT	GGAAACCAAG	TTTGATTTG	CCATGAAGTT	CAATGCACTG	1320
GAACCTGGATG	ACAGTGTAT	CTCCCCTTTT	GTGGCTGCTA	TCATTTGCTG	TGGAGATCGT	1380
CCTGGCCTTC	TAACAGTAGG	ACACATTGAA	AAAATGCAGG	AGGGTATTGT	ACATGTGCTC	1440
AGACTCCACC	TGCAAGAGCAA	CCACCCGGAC	GATATCTTTC	TCTTCCCAA	ACTTCTTCAA	1500
AAAATGGCAG	ACCTCCGGCA	GCTGGTGACG	GAGCATGCGC	AGCTGGTGCA	GATCATCAAG	1560
AAGACGGAGT	CGGATGCTGC	GTCGACCCG	CTACTGCAGG	AGATCTACAG	GGACATGTAC	1620
TGAGTCCCTT	CAGATCAGCC	ACACCTTTTC	CAGGAGTTCT	GAAGCTGACA	GCACTACAA	1680
GGAGACGGGG	GAGCAGCACG	ATTTTGACAA	AATATCCACC	ACTTTAACCT	TAGAGCTTGG	1740
ACAGTCTGAG	CTGTAGGTA	CGGGCATATT	ATTCCATATC	TTTGTGTTAA	CCACTACTTC	1800
TAAGAGCATA	GAACTCAAAT	GCTGGGGAG	GTGGCTAATC	TCAGGACTGG	GAAG	1854

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 478 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met	Thr	Met	Val	Asp	Thr	Glu	Ile	Ala	Phe	Trp	Pro	Thr	Asn	Phe	Gly
1														15	
															5

Ile Ser Ser Val Asp Leu Ser Val Met Glu Asp His Ser His Ser Phe
 20 25 30
 Asp Ile Lys Pro Phe Thr Thr Val Asp Phe Ser Ser Ile Ser Thr Pro
 35 40 45
 His Tyr Glu Asp Ile Pro Phe Thr Arg Thr Asp Pro Val Val Ala Asp
 50 55 60
 Tyr Lys Tyr Asp Leu Lys Leu Gln Glu Tyr Gln Ser Ala Ile Lys Val
 65 70 75 80
 Glu Pro Ala Ser Pro Pro Tyr Tyr Ser Glu Lys Thr Gln Leu Tyr Asn
 85 90 95
 Lys Pro His Glu Glu Pro Ser Asn Ser Leu Met Ala Ile Glu Cys Arg
 100 105 110
 Val Cys Gly Asp Lys Ala Ser Gly Phe His Tyr Gly Val His Ala Cys
 115 120 125
 Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr Ile Arg Leu Lys Leu Ile
 130 135 140
 Tyr Asp Arg Cys Asp Leu Asn Cys Arg Ile His Lys Lys Ser Arg Asn
 145 150 155 160
 Lys Cys Gln Tyr Cys Arg Phe Gln Lys Cys Leu Ala Val Gly Met Ser
 165 170 175
 His Asn Ala Ile Arg Phe Gly Arg Ile Ala Gln Ala Glu Lys Glu Lys
 180 185 190
 Leu Leu Ala Glu Ile Ser Ser Asp Ile Asp Gln Leu Asn Pro Glu Ser
 195 200 205
 Ala Asp Leu Arg Gln Ala Leu Ala Lys His Leu Tyr Asp Ser Tyr Ile
 210 215 220
 Lys Ser Phe Pro Leu Thr Lys Ala Lys Ala Arg Ala Ile Leu Thr Gly
 225 230 235 240
 Lys Thr Thr Asp Lys Ser Pro Phe Val Ile Tyr Asp Met Asn Ser Leu
 245 250 255
 Met Met Gly Glu Asp Lys Ile Lys Phe Lys His Ile Thr Pro Leu Gln
 260 265 270
 Glu Gln Ser Lys Glu Val Ala Ile Arg Ile Phe Gln Gly Cys Gln Phe
 275 280 285
 Arg Ser Val Glu Ala Val Gln Glu Ile Thr Glu Tyr Ala Lys Ser Ile
 290 295 300
 Pro Gly Phe Val Asn Leu Asp Leu Asn Asp Gln Val Thr Leu Leu Lys
 305 310 315 320
 Tyr Gly Val His Glu Ile Ile Tyr Thr Met Leu Ala Ser Leu Met Asn
 325 330 335
 Lys Asp Gly Val Leu Ile Ser Glu Gly Gln Gly Phe Met Thr Arg Glu
 340 345 350
 Phe Leu Lys Ser Leu Arg Lys Pro Phe Gly Asp Phe Met Glu Pro Lys
 355 360 365
 Phe Glu Phe Ala Val Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp
 370 375 380
 Leu Ala Ile Phe Ile Ala Val Ile Ile Leu Ser Gly Asp Arg Pro Gly
 385 390 395 400
 Leu Leu Asn Val Lys Pro Ile Glu Asp Ile Gln Asp Asn Leu Leu Gln
 405 410 415
 Ala Leu Glu Leu Gln Leu Lys Leu Asn His Pro Glu Ser Ser Gln Leu
 420 425 430
 Phe Ala Lys Leu Leu Gln Lys Met Thr Asp Leu Arg Gln Ile Val Thr
 435 440 445
 Glu His Val Gln Leu Leu Gln Val Ile Lys Lys Thr Glu Thr Asp Met
 450 455 460

Ser Leu His Pro Leu Leu Gln Glu Ile Tyr Lys Asp Leu Tyr
 465 470 475

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1811 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCGACCTTAC	CCCAGGGCGC	CTTGACGTTG	GTCTTGTGCG	CAGGAGACAG	CACCATGGTG	60
GGTCTCTCT	GAGTCCTGGGA	ATTCGGGAGC	CCGAGCCGCA	GCCGCCGCCT	GGGGGGCTTG	120
GGTCGGCCTC	GAGGACACCG	GAGAGGGCG	CCACGCCGCC	GTGGCCGCAG	AAATGACCAT	180
GGTTGACACA	GAGATCGCAT	TCTGGCCAC	CAACTTTGGG	ATCAGCTCCG	TGGATCTCTC	240
CGTAATGGAA	GACCACTCCC	ACTCCTTGA	TATCAAGCCC	TTCACTACTG	TTGACTTCTC	300
CAGCATTCTC	ACTCCACATT	ACGAAGACAT	TCCATTCACA	AGAACAGATC	CAGTGGTTGC	360
AGATTACAAG	TATGACCTGA	AACTTCAGA	GTACCAAAGT	GCAATCAAAG	TGGAGCCTGC	420
ATCTCCACCT	TATTATTCTG	AGAACACTCA	GCTCTACAAAT	AAGCTCTCATG	AAGAGCCTTC	480
CAACTCCCTC	ATGGCAATTG	AATGCTGTTG	CTGTGGAGAT	AAAGCTTCTG	GATTTCACTA	540
TGGAGTTCAT	GCTTGTGAAG	GATGCAAGG	TTTCTTCCGG	AGAACAAATC	GATTGAAGCT	600
TATCTATGAC	AGATGTGATC	TTAACTGTG	GATCCACAAA	AAAAGTAGAA	ATAATGTCA	660
GTACTGTCGG	TTTCAGAAAT	GCCTTGCAGT	GGGGATGTCT	CATAATGCCA	TCAGTTTGG	720
GCGGATCGCA	CAGGCGGAGA	AGGAGAAAGT	GTGCGGGAG	ATCTCAGTG	ATATCGACCA	780
GCTGAATCCA	GAGTCGGCTG	ACCTCCGTC	GGCCCTGGCA	AAACATTTGT	ATGACTCATA	840
CATAAAAGTCC	TTCCCCGCTGA	CCAAAGCAAA	GGCGAGGGCG	ATCTTGACAG	GAAAGACAAC	900
AGACAAATCA	CCATTGCTTA	TCTATGACAT	GAATTCTTA	ATGATGGGAG	AAGATAAAAT	960
CAAGTCAAA	CACATCACCC	CCCTGCAGGA	GCAGAGCAAA	GAGGTGGCCA	TCCGCATTT	1020
TCAGGGCTGC	CAGTTTCGCT	CCGTGGAGGC	TGTCCAGGAG	ATCACAGAGT	ATGCCAAAAG	1080
CATTCTCTGGT	TTTGTAAATC	TTGACTTGA	CGACCAAGTA	ACTCTCTCTA	AAATATGGAGT	1140
CCACGAGATC	ATTACACAA	TGCTGGCTC	CTTGTATGAAT	AAAGATGGGG	TTCTCATATC	1200
CGAGGGCCAA	GGCTTCATGA	CAAGGGAGT	TCTAAAGAGC	CTGGCAAAGC	CTTTTGGTGA	1260
CTTTATGGAG	CCCAAGTTTG	AGTTTGCTGT	GAAGTTCAAT	GCACCTGGAAT	TAGATGACAG	1320
CGACTTGGCA	ATATTTTATTG	CTGTCATTAT	TCTCAGTGG	GACCGCCCG	GTTPGCTGAA	1380
TGTGAAGCCC	ATTGAAGACA	TTCAAGACAA	CCTGTACAA	GCCCTGGAGC	TCCAGCTGAA	1440
GCTGAACCAC	CCTGAGTCCT	CACAGCTGTT	TGCCAAGCTG	CTCCAGAAAA	TGACAGACCT	1500
CAGACAGATT	GTCACGGAAC	ACGTGCAGCT	ACTGCAGGTG	ATCAAGAAGA	CGGAGACAGA	1560
CATGAGTCCT	CACCCGCTCC	TGCAGGAGAT	CTACAAGGAC	TTGTACTAGC	AGAGAGTCCT	1620
GAGCCACTGC	CAACATTTC	CTTCTTCCAG	TTGCACTATT	CTGAGGGAAA	ATCTGACCAT	1680
AAGAAAATTA	CTGTAAAAAA	GCGTTTAA	AAGAAAAGGG	TTTAGAATAT	GATCTATTTT	1740
ATGCATATTG	TTTATAAAGA	CACATTACA	ATTACTTTT	AATATTAAAA	ATTACCATAT	1800
TATGAAATTG	C					1811

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 441 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Glu Gln Pro Gln Glu Ala Pro Glu Val Arg Glu Glu Glu
 1 5 10 15
 Lys Glu Glu Val Ala Glu Ala Glu Gly Ala Pro Glu Leu Asn Gly Gly
 20 25 30
 Pro Gln His Ala Leu Pro Ser Ser Ser Tyr Thr Asp Leu Ser Arg Ser
 35 40 45
 Ser Ser Pro Pro Ser Leu Leu Asp Gln Leu Gln Met Gly Cys Asp Gly
 50 55 60
 Ala Ser Cys Gly Ser Leu Asn Met Glu Cys Arg Val Cys Gly Asp Lys
 65 70 75 80
 Ala Ser Gly Phe His Tyr Gly Val His Ala Cys Glu Gly Cys Lys Gly
 85 90 95
 Phe Phe Arg Arg Thr Ile Arg Met Lys Leu Glu Tyr Glu Lys Cys Glu
 100 105 110
 Arg Ser Cys Lys Ile Gln Lys Lys Asn Arg Asn Lys Cys Gln Tyr Cys
 115 120 125
 Arg Phe Gln Lys Cys Leu Ala Leu Gly Met Ser His Asn Ala Ile Arg
 130 135 140
 Phe Gly Arg Met Pro Glu Ala Glu Lys Arg Lys Leu Val Ala Gly Leu
 145 150 155 160
 Thr Ala Asn Glu Gly Ser Gln Tyr Asn Pro Gln Val Ala Asp Leu Lys
 165 170 175
 Ala Phe Ser Lys His Ile Tyr Asn Ala Tyr Leu Lys Asn Phe Asn Met
 180 185 190
 Thr Lys Lys Ala Arg Ser Ile Leu Thr Gly Lys Ala Ser His Thr
 195 200 205
 Ala Pro Phe Val Ile His Asp Ile Glu Thr Leu Trp Gln Ala Glu Lys
 210 215 220
 Gly Leu Val Trp Lys Gln Leu Val Asn Gly Leu Pro Pro Tyr Lys Glu
 225 230 235 240
 Ile Ser Val His Val Phe Tyr Arg Cys Gln Cys Thr Thr Val Glu Thr
 245 250 255
 Val Arg Glu Leu Thr Glu Phe Ala Lys Ser Ile Pro Ser Phe Ser Ser
 260 265 270
 Leu Phe Leu Asn Asp Gln Val Thr Leu Leu Lys Tyr Gly Val His Glu
 275 280 285
 Ala Ile Phe Ala Met Leu Ala Ser Ile Val Asn Lys Asp Gly Leu Leu
 290 295 300
 Val Ala Asn Gly Ser Gly Phe Val Thr Arg Glu Phe Leu Arg Ser Leu
 305 310 315 320
 Arg Lys Pro Phe Ser Asp Ile Ile Glu Pro Lys Phe Glu Phe Ala Val
 325 330 335
 Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp Leu Ala Leu Phe Ile
 340 345 350
 Ala Ala Ile Ile Leu Cys Gly Asp Arg Pro Gly Leu Met Asn Val Pro
 355 360 365
 Arg Val Glu Ala Ile Gln Asp Thr Ile Leu Arg Ala Leu Glu Phe His
 370 375 380
 Leu Gln Ala Asn His Pro Asp Ala Gln Tyr Leu Phe Pro Lys Leu Leu
 385 390 395 400
 Gln Lys Met Ala Asp Leu Arg Gln Leu Val Thr Glu His Ala Gln Met
 405 410 415

Met Gln Arg Ile Lys Lys Thr Glu Thr Glu Thr Ser Leu His Pro Leu
 420 425 430
 Leu Gln Glu Ile Tyr Lys Asp Met Tyr
 435 440

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3301 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAATTCTGCG	GAGCCCTGCGG	GACGGGGCGG	GGTTGGCCG	TAGGCAGCCG	GGACAGTGT	60
GTACAGTGT	TIGGGCATGC	ACGTGATACT	CACACAGTGG	CTTCTGCTCA	CCAACAGATG	120
AAGACAGATG	CACCAACAGG	GGTCTGGAAT	GGTCTGGAGT	GGTCTGGAAA	GCAGGGTCAG	180
ATACCCCTGG	AAAATGAAG	CCCGTGGAGC	AATGATCTCT	ACAGGACTG	TTCAAGGCTG	240
ATGGGAACCA	CCCTGTAGAG	GTCCCATCTGC	GTTCAGACCC	AGACGATGCC	AGAGCTATGA	300
CTGGGGCTGC	AGGTGTGGCG	CCGGAGGGAG	ATCACGCCATG	GAGCAGCCAC	AGGAGGAAGC	360
CCCTGAGGTC	CGGGAAGAGG	AGGAGAAAAGA	GGAAGTGGCA	GAGGCAGAAAG	GAGCCCAGA	420
GCTCAATGGG	GGACCACAGC	ATGCACTTCC	TTCCAGCAGC	TACACAGACC	TCTCCGGAG	480
CTCCCTGCCA	CCCTCACTGC	TGGACCAAAT	GCAGATGGGC	TGTGACGGGG	CCTCATGCGG	540
CAGCCTCAAC	ATGGAGTGCC	GGGTGTGCGG	GGACAAGGCA	TGGGGCTTCC	ACTACGGTGT	600
TCATGCATGT	GAGGGGTGCA	AGGGCTTCTT	CCGTCGTACG	ATCCGCATGA	ACCTGGAGTA	660
CGAGAACGTG	GACCGCAGCT	GCAAGATTCGA	GAAGAAGAAC	CGCAACAAGT	GCCAGTACTG	720
CCGCTTCCAG	AAGTGCCTGG	CACTGGGCAT	GTCACACAAC	GCTATCCGTT	TTGGTCGGAT	780
GCGGGAGGCT	GAGAACAGGA	AGCTGGTGGC	AGGGCTGACT	GCAAACGAGG	GGAGCCAGTA	840
CAACCCACAG	GTGCCGACCC	TGAAGGCCCT	CTCCAAGCAC	ATCTACAATG	CCTACCTGAA	900
AAACATTCAAC	ATGACCAAAA	AGAAGGCCCG	CAGCATCCTC	ACCGGCAAAG	CCAGCCACAC	960
GGGCCCTTTT	GTGATCCACG	ACATCGAGAC	ATTGTTGGCAG	GCAGAGAAGG	GGCTGGTGTG	1020
GAAGCAGTTG	GTGAATGGCC	TGCCCTCCCTA	CAAGGAGATC	AGCGTGCACG	TCTTCTACCG	1080
CTGCCAGTGC	ACACACAGTGG	AGACCGTGCG	GGAGCTCACT	GAGTTCGCCA	AGAGCATCCC	1140
CAGCTTCAGC	AGCCTCTTCC	TCAACGCCA	GGTTACCCCTT	CTCAAGTATG	GGGTGACCGA	1200
GGCCATCTTC	GCCATGCTGG	CCTCTATCGT	CAACAAGGAC	GGGCTGCTGG	TAGCCAACGG	1260
CAGTGGCTTT	GTCACCCCGTG	AGTTCTGCG	CAGCCTCCCG	AAACCCCTCA	GTGATATCAT	1320
TGAGCCTAAG	TTTGAAATTG	CTGTCAGTT	CAACGCCCTG	GAACCTGATG	ACAGTGACCT	1380
GGCCCTTATTG	ATTGCGGCCA	TCATTCTGTG	TGGAGACCGG	CCAGGCCCTCA	TGAACGTTCC	1440
ACGGGTGGAG	GCTATCCAGG	ACACCATCCT	GGGTGCCCTC	GAATTCCACC	TGCAGGCCAA	1500
CCACCCCTGAT	GCCCCAGTACC	TCTTCCCCAA	GCTGCTGCAG	AAGATGGCTG	ACCTGGGCA	1560
ACTGGTCACC	GAGCACGCC	AGATGATGCA	GCGGATCAAG	AAGACCGAAA	CCGAGACCTC	1620
GCTGCCACCT	CTGCTCCAGG	AGATCTACAA	GGACATGTAC	TAACGGCCGC	ACCCAGGCCT	1680
CCCTGCGAC	TCCAATGGGG	CCAGCACTGG	AGGGGCCAC	CCACATGACT	TTTCCATTGA	1740
CCAGCTCTCT	TCCGTCTTT	GTTGTCCTCC	TCTTCTCAG	TTCCCTTTTC	TTTTCTAATT	1800
CCTGTTGCTC	TGTTTCTTCC	TTTCTGTAGG	TTTCTCTCTT	CCCTTCTCCC	TTCTCCCTTG	1860
CCCTCCCTTT	CTCTCTCCTA	TCCCCACGTC	TGTCCTCTT	TCTTATTCTG	TGAGATGTTT	1920
TGTATTATTT	CACCAAGCAGC	ATAGAACAGG	ACCTCTGCTT	TTGCACACCT	TTTCCCCAGG	1980
AGCAGAACAG	AGTGGGCCTG	CCCTCTGCC	CATCATGCA	CCTGCAGGCT	TAGGTCCTCA	2040
CTTCTGTCTC	CTGCTCTCAG	AGCAAAAGAC	TTGAGCCATC	CAAAGAAACA	CTAACGCTCTC	2100
TGGGCCTGGG	TTCCAGGGAA	GGCTAACCAT	GGCCTGGACT	GAATGCAGCC	CCCTATAGTC	2160
ATGGGGTCCC	TGCTGCAAAG	GACAGTGGCA	GACCCCGGCA	GTAGAGCCGA	GATGCCCTCCC	2220
CAAGACTGTC	ATTGCCCCCTC	CGATCGTGAG	GCCACCCACT	GACCCAAATGA	TCCTCTCCAG	2280
CAGCACACCT	CAGCCCCACT	GACACCCAGT	GTCCCTCCAT	CTTCACACTG	GTTTGGCCAGG	2340

CCAATGTTGC	TGATGGCCCC	TCCAGCACAC	ACACATAAGC	ACTGAAATCA	CTTACCTGC	2400
AGGCACCATG	CACCTCCCTT	CCCTCCCTGA	GGCAGGTGAG	AACCCAGAGA	GAGGGGCCCTG	2460
CAGGTGAGCA	GGCAGGGCTG	GGCCAGGTCT	CCGGGGAGGC	AGGGGTCTG	CAGGTCTGG	2520
TGGGTCAAGCC	CACCACTCG	CCCAGTGGGA	GCTTCCCGGG	ATAAACTGAG	CCTGTTCTATT	2580
CTGATGTCCA	TTTGCCCCAA	TAGCTCTACT	GGCCCTCCCT	TCCCCTTTAC	TCAGCCCAGC	2640
TGGCCACCTA	GAAGTCTCCC	TGCACAGCCT	CTAGTGTCCG	GGGACCTTGT	GGGACCAAGTC	2700
CCACACCGCT	GGTCCTGCC	CTCCCCCTGCT	CCCAGGTTGA	GGTGCCTCA	CCTCAGAGCA	2760
GGGCCAAAGC	ACAGCTGGC	ATGCCATGTC	TGAGCGGCGC	AGAGCCCTCC	AGGCCTGCAG	2820
GGGCAAGGGG	CTGGCTGGAG	TCTCAGAGCA	CAGAGGTAGG	AGAAACTGGGG	TTCAACCCCCA	2880
GGCTTCCCTGG	GTCCCTGCCG	GTCCCTCCCTC	CCAAGGAGCC	ATTCTATGTG	ACTCTGGGTG	2940
GAAGTGCCTCA	GCCCCCTGCC	GACGGNNNNN	NNGATCACTC	TCTGCTGGCA	GGATTCTTCC	3000
CGCTCCCCAC	CTACCCAGCT	GATGGGGGTT	GGGGTGCCTC	TTTCAGCCAA	GGCTATGAAG	3060
GGACAGCTGC	TGGGACCCAC	CTCCCCCCTT	CCCCGGCCAC	ATGCCGCGTC	CCTGCCCCCA	3120
CCCGGGTCTG	GTGCTGAGGA	TACAGCTCTT	CTCAGTGTCT	GAACAATCTC	AAAAATTGAA	3180
ATGTATATTT	TTGCTAGGAG	CCCCAGCTTC	CTGTGTTTTT	AATATAAATA	GTGTACACAG	3240
ACTGACGAAA	CTTTAAATAA	ATGGGAATT	AATATTAAA	AAAAAAAGCG	GCCGCGAATT	3300
C						3301

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACTCGGATCC AAGCCATGGC TGAGAACTTG CTGGACGG

38

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 32 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CACAAAGCTT AGGCCATGTT AGCACTGTTC GG

32

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 37 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

WO 99/18124

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTCAGTCGAC TTATTGAATT CCACTAGCTG GAGATCC

37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21049

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/435, 14/705; C09K 11/06; G01N 33/53, 33/566
US CL :252/301.16, 301.36, 301.4R; 435/7.8; 530/350, 358

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 252/ 301.16, 301.32, 301.4R; 435/7.8; 530/350, 358

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	ZHOU et al. Nuclear receptors have distinct affinities for coactivators: characterization by fluorescence resonance energy transfer. Molecular Endocrinology. October 1998. Vol. 12, No. 10, pages 1594-1604, especially page 1596 and figures 1-4.	1-27

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
 - *B* earlier document published on or after the international filing date
 - *L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 - *O* document referring to an oral disclosure, use, exhibition or other means
 - *P* document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "a." document member of the same patent family

Date of the actual completion of the international search

26 DECEMBER 1998

Date of mailing of the international search report

21 JAN 1999

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/21049

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN, BIOSCIENCE, BIOSIS, CAPLUS, SCISEARCH

search terms: nuclear receptor, steroid receptor, retinoic acid receptor, co-activator, fref